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DISTRIBUTION OF *LUCIFER FAXONI* (CRUSTACEA: DECAPODA: SERGESTIDAE) IN NERITIC WATERS OFF THE TEXAS COAST, WITH A NOTE ON THE OCCURRENCE OF *LUCIFER TYPUS*

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ABSTRACT

Seasonal variation in abundance was exhibited by *Lucifer faxoni* in Texas neritic waters in 1962; the population increased in nearshore waters in August—September and declined in October—November. During the remainder of the year the animals were dispersed widely over the study area. Large numbers of larvae in peak populations suggest that August—November is the period of maximum reproductive activity of *L. faxoni*. Larval abundance decreased markedly beyond the nearshore (14 m) stations, indicating that reproduction is confined primarily to nearshore waters. Water movements in the study area combined with the vertical migration pattern of *L. faxoni* may be instrumental in initiating the population increase by concentrating adults in nearshore water, facilitating mating. Population density may be related to increasing water temperature and the autumnal phytoplankton bloom. The occurrence of *Lucifer typus* in Texas neritic waters is noted.

INTRODUCTION

Lucifer faxoni Borradaile (1915), one of the relatively few holoplanktonic decapod crustaceans, is a member of the neritic plankton along the eastern coasts of North and South America from Long Island Sound, New York to Rio de

Janeiro, Brazil. The known distribution of *L. faxoni* was summarized by Bowman and McCain (1967).

Prior reports by several authors (Arnold, Wheeler and Baxter 1960; Burkenroad 1934; Grice 1956; Hopkins 1966; Kelly and Dragovich 1967; Tabb and Manning 1961; Wass 1955; Woodmansee 1966) have indicated that *Lucifer faxoni* is distributed continuously along the U.S. Gulf Coast from Florida Bay to Galveston, Texas. Without specifying the source of their information, Bowman and McCain (1967) noted the occurrence of *L. faxoni* in the vicinities of Brownsville, Texas and Vera Cruz, Mexico.

The seasonal distribution of *Lucifer* has been studied or briefly noted at several localities. Investigators reporting population abundances (Deevey 1960; Hopkins 1966; Kelly and Dragovich 1967) agree that maximum populations occur in the summer and fall, with lowest populations during winter. Arnold *et al.* (1960) reported occurrence of *L. faxoni* in Galveston Bay and noted that it was not collected in winter.

Available bathymetric distributional records of *Lucifer faxoni* indicate that the plankter is most abundant in nearshore waters and near the mouths of bays. Populations generally decrease towards the heads of estuaries and with increasing depth offshore (Hopkins 1966; Kelly and Dragovich 1967; Woodmansee 1966). Similar patterns were reported by Davis (1950) and King (1950) for an unidentified species of *Lucifer* which has been attributed to be *L. faxoni*.

Because most plankton studies in which *Lucifer* has been reported have dealt with large numbers of organisms, the investigators have not been able to make detailed studies of the distribution of individual species. This study was concerned with only one member of the planktonic community off the Texas Gulf Coast, and as a result, seasonal and bathymetric distributions of *Lucifer* were investigated in greater detail than in prior papers. Particular effort was made to correlate distributional patterns with environmental factors.

Plankton for this study was collected by the U.S. Fish and Wildlife Service, Galveston, Texas at stations along three transects normal to the Texas coast (Fig. 1). Samples were taken at monthly intervals from January through December 1962.

FIELD PROCEDURES

Samples were taken with a Gulf V plankton sampler (Arnold 1959). Step-oblique tows were made, commencing 3 meters from the bottom and terminating 3 meters from the surface. Samples preserved in 10% formalin aboard ship were later reduced and stored in 5% buffered formalin. The number of revolutions of a propeller-driven meter at the front of the sampler was used to calculate the volume of water filtered.

Temperature and salinity were determined at 3 to 7 depths, depending on the total depth of a given station. Through June 1962, determinations were made by a Foxboro Dynalog; thereafter temperatures were recorded by a bathythermograph and salinities determined by Knudsen titration of water samples taken with Nansen bottles. No temperature or salinity data were taken in May.

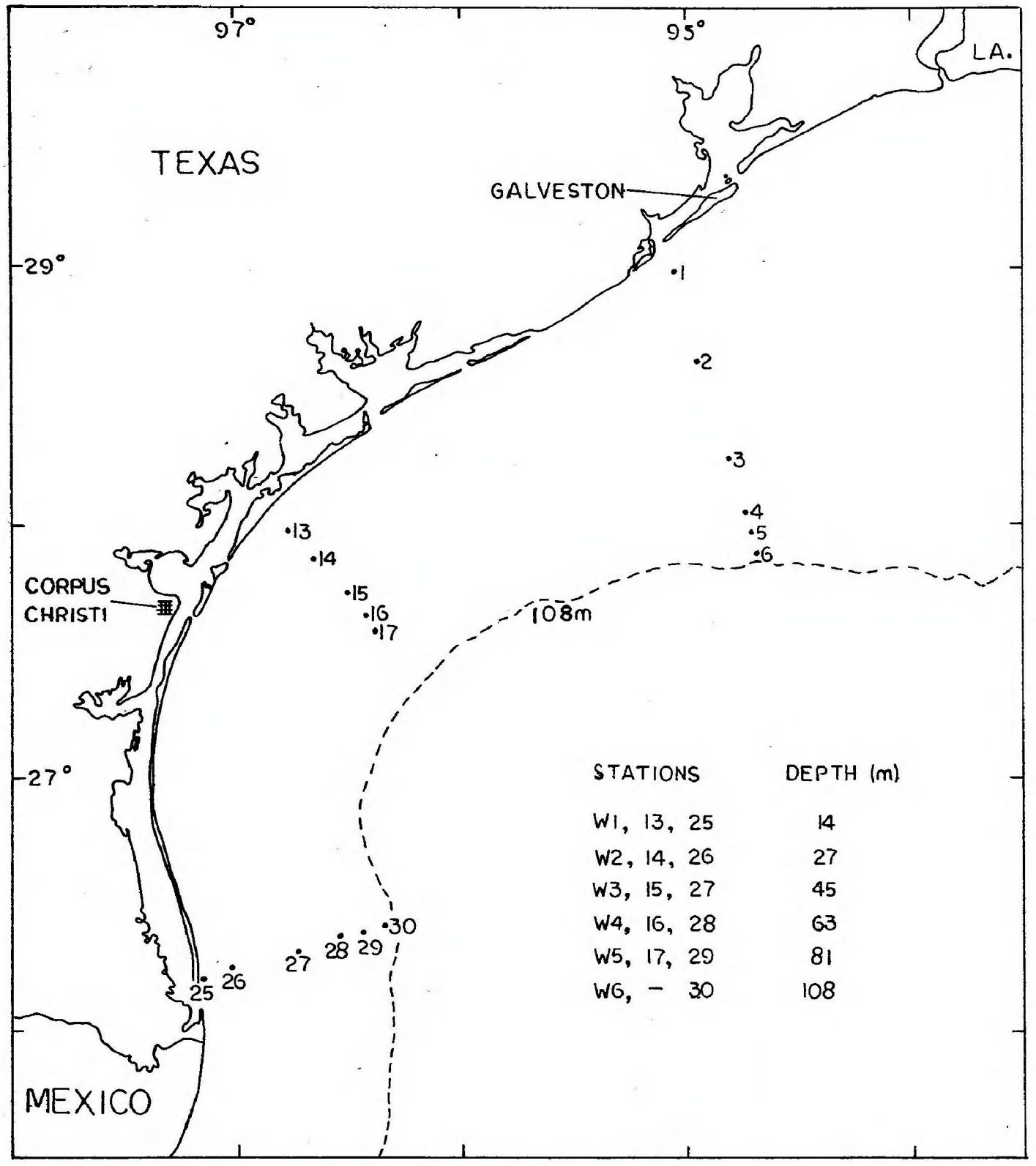

FIG. 1. Map of the study area showing stations at which samples were taken in 1962.

LABORATORY PROCEDURES

Initially, monthly samples for three inshore stations, W1, W13 and W25, and their corresponding offshore stations, W6, W17 and W30, were examined to gain insight into seasonal and bathymetric distribution of *Lucifer*. A marked difference between inshore and offshore populations was found only in the fall, so only samples collected at the intermediate stations in the fall were examined to determine bathymetric distribution.

All specimens of *Lucifer* were removed from one-fifth portions (*ca.* 48 ml) of the stored samples. The animals were sexed, examined for development of eggs,

spermatophores and secondary sexual characteristics, and the stage of development noted. The number of each stage was related to the volume of water strained and reported as the number per 100 m^3. The zoeae were very abundant in fall samples, making it impractical to remove all of them. To calculate the number of zoeae, five 1 ml aliquots of the 48 ml portions were examined. The mean number of zoeae in the aliquots was related to the sample volume to estimate the number in the sample, and the number per 100 m^3 was then calculated. No nauplii were noted in any samples; their small size (approximately the same as the Gulf V mesh opening; *i.e.*, 0.22 mm) probably allowed most to slip through the net.

During the study, seven categories of *Lucifer* were used to correlate developmental stage abundances with temperature and salinity; *i.e.*, adult male, adult female, young male, young female, postlarva, mysis and zoea. In this report, the first four categories are referred to as adults, and the last three categories as larvae.

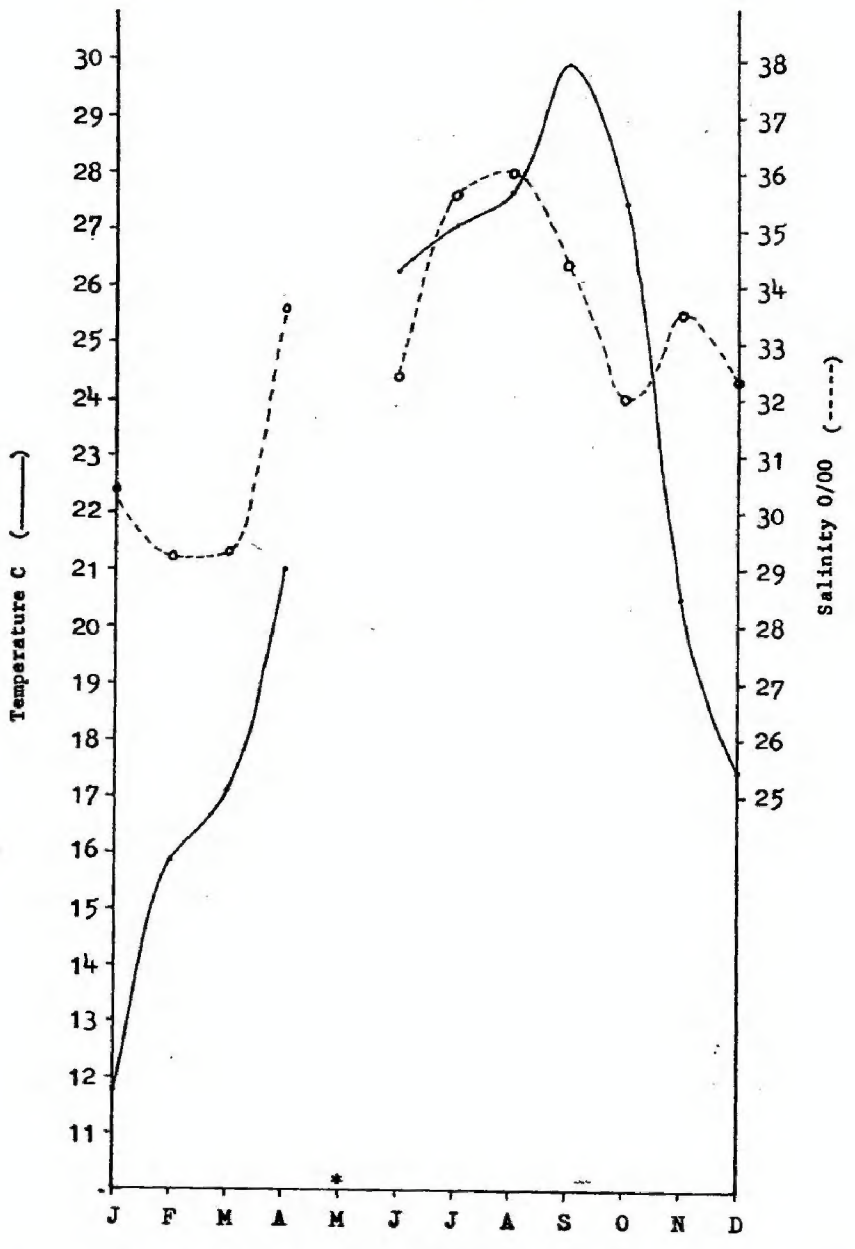

FIG. 2. Average monthly temperatures and salinities of the water column at station W1. *—denotes no data taken.

HYDROGRAPHY

Average monthly temperatures and salinities of the water columns were calculated and plotted for each 14 m station (Figs. 2, 3, 4). The minimum inshore temperature was 11.8 C in January, and the maximum was 30.0 C in September, both at W1. Temperature profiles of the three stations corresponded well with each other from January through June. Thereafter, temperatures at W1 and W13 were similar, reaching maxima in September and declining rapidly through December. Temperatures increased more slowly at W25 than at W1 or W13, reaching a maximum in October, but declined more slowly than temperatures at W1 and W13.

The minimum salinity was 32.0 ppt at W1 in October; the maximum was 37.3 ppt at W13 in July. Salinity fluctuations at the three stations were similar, with the salinities increasing to a maximum in August or September and then declining through December. Salinities at W25 generally were 2–4 ppt higher than at W1 or W13.

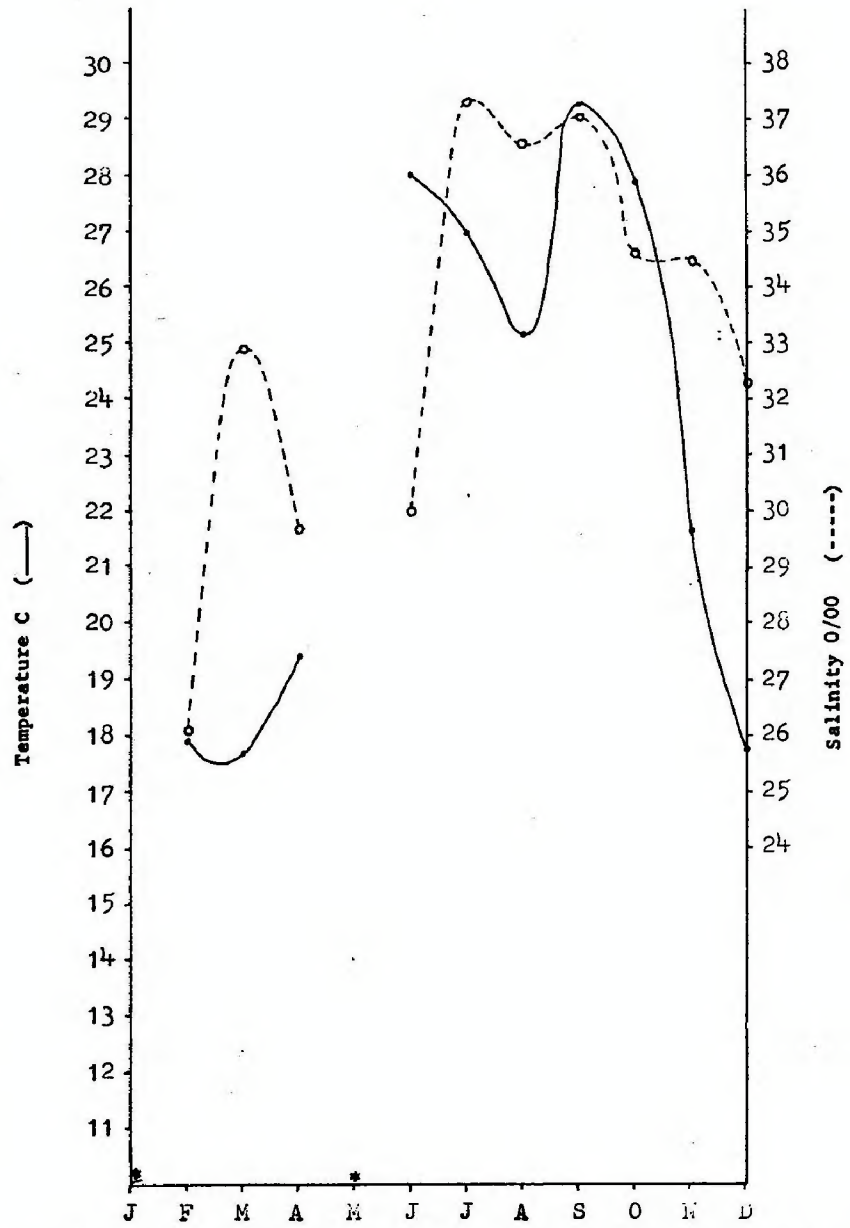

FIG. 3. Average monthly temperatures and salinities of the water column at station W13. *—denotes no data taken.

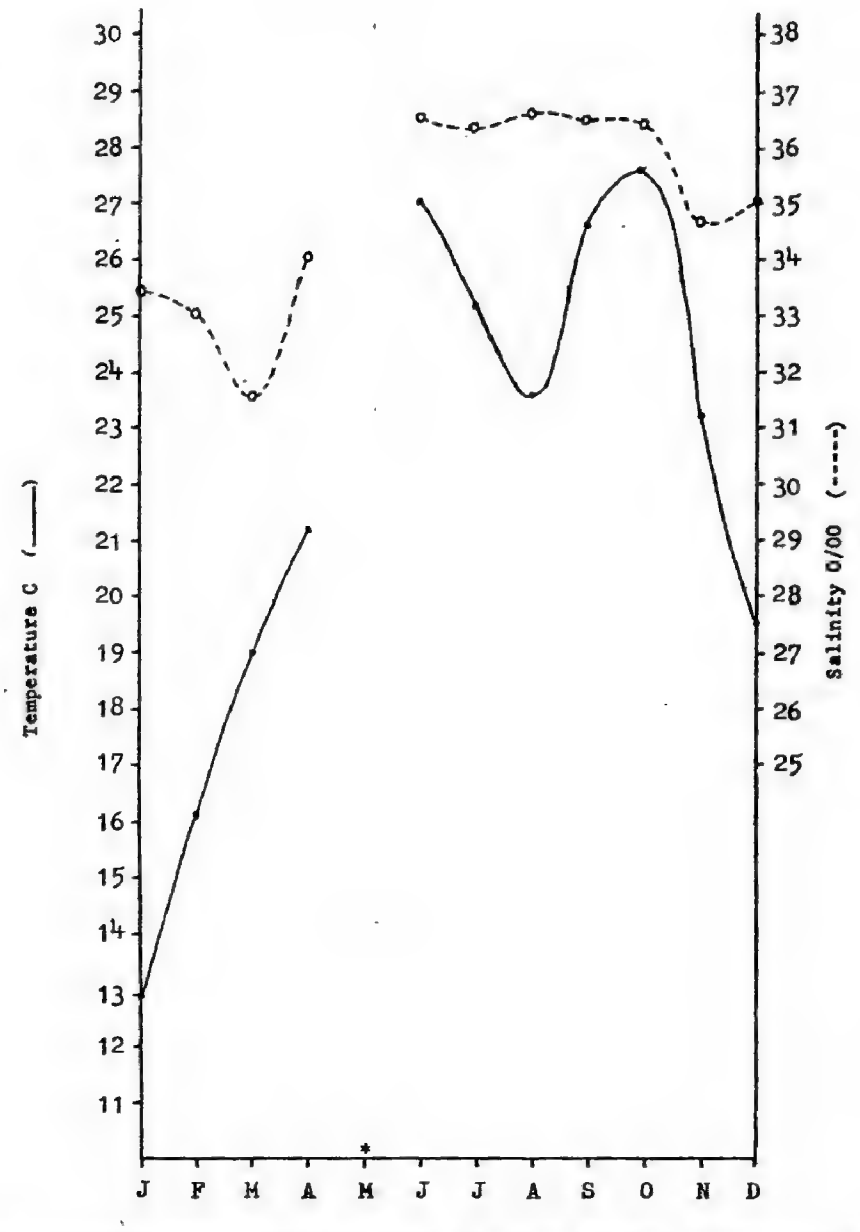

FIG. 4. Average monthly temperatures and salinities of the water column at station W25. *—denotes no data taken.

SEASONAL DISTRIBUTION OF *LUCIFER FAXONI*

Comparisons of monthly populations at corresponding inshore and offshore stations (Figs. 5, 6, 7) indicate that *Lucifer* was present in small numbers from January to mid-summer or early fall at all stations. An increase in population occurred inshore in late summer or early fall, an apparent lag in the increase occurring at successively westerly stations. The peak population lasted 2 to 3 months, then declined by late fall and remained low through December.

In comparison, concentrations of organisms at offshore stations (108 m) never approached the large numbers found at their inshore counterparts in the fall. Aside from the inshore maxima in the fall, the animals were dispersed widely at inshore and offshore stations during the remainder of the year. Thus, temporal distribution of *L. faxoni* along the Texas coast corresponds closely with data obtained by other investigators (Deevey 1960; Hopkins 1966; Kelly and Dragovich 1967), all of whom found maximum populations in summer and fall.

The large inshore fall peaks in *Lucifer* populations were due primarily to the presence of many larval stages in the plankton (Figs. 8, 9, 10). Fall populations of *L. faxoni* decreased markedly seaward of the 14 meter stations (Figs. 11, 12,

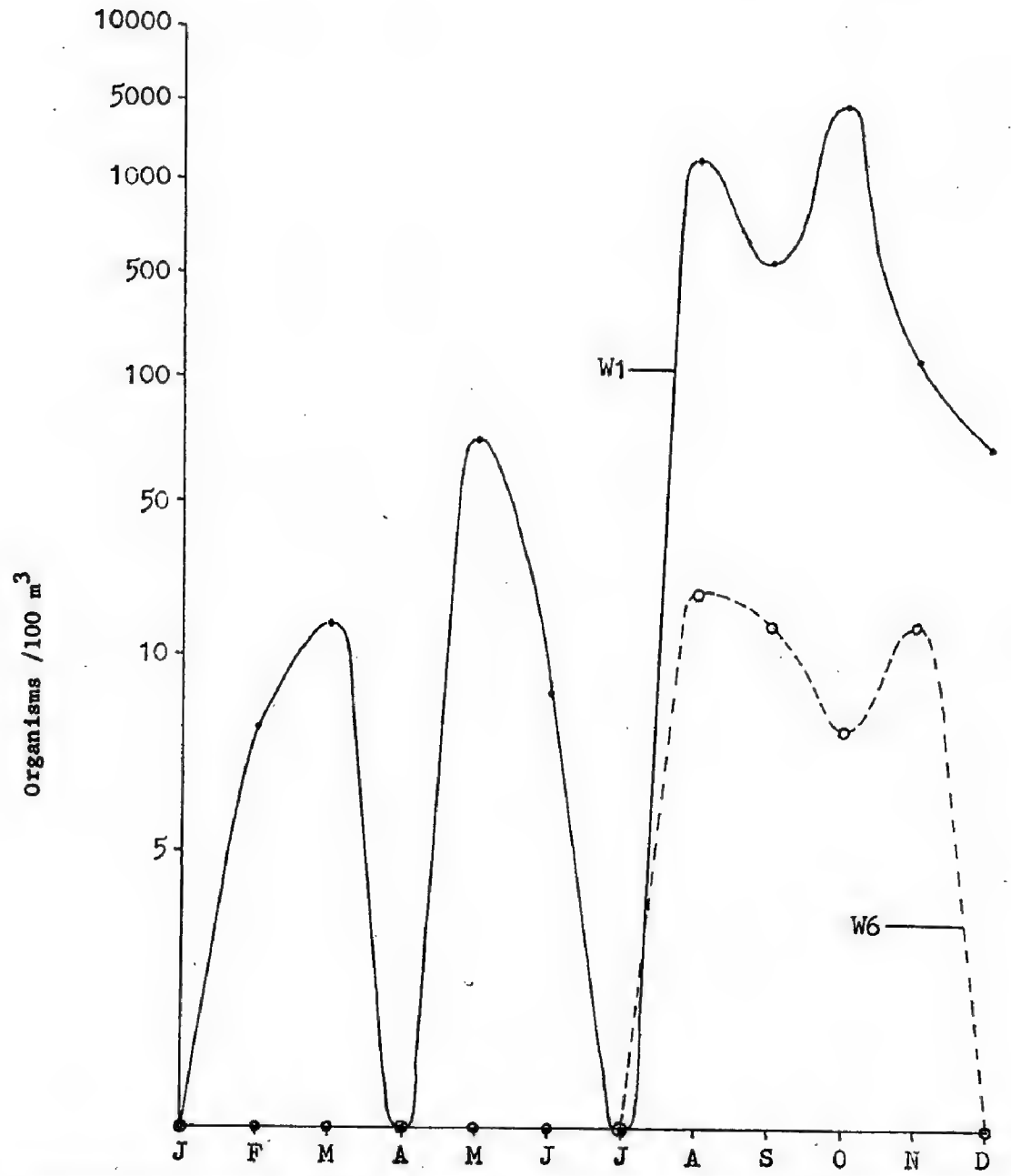

FIG. 5. Comparative seasonal distribution of *L. faxoni* at stations W1 (14 m) and W6 (108 m) plotted on a semi-log scale.

13). The decrease was chiefly due to a great reduction in larval abundance. Bathymetric distribution of *Lucifer* off the Texas coast compares favorably with reports by Davis (1950), King (1950) and Woodmansee (1966).

OCCURRENCE OF *LUCIFER* IN RELATION TO TEMPERATURE AND SALINITY

A method similar to that used by Kelly and Dragovich (1967) was employed to relate inshore occurrences of *L. faxoni* with water temperatures. The average monthly temperatures of the 14 meter stations' water columns were divided into three ranges, each range including about an equal number of temperature observations. The numbers of adults, young and larvae associated with each temperature range were calculated and expressed as a percentage of the total number of organisms collected during the year. The relationship between salinity and occurrence of *Lucifer* was similarly determined.

A comparison of occurrence of *L. faxoni* vs. temperature and salinity (Table 1)

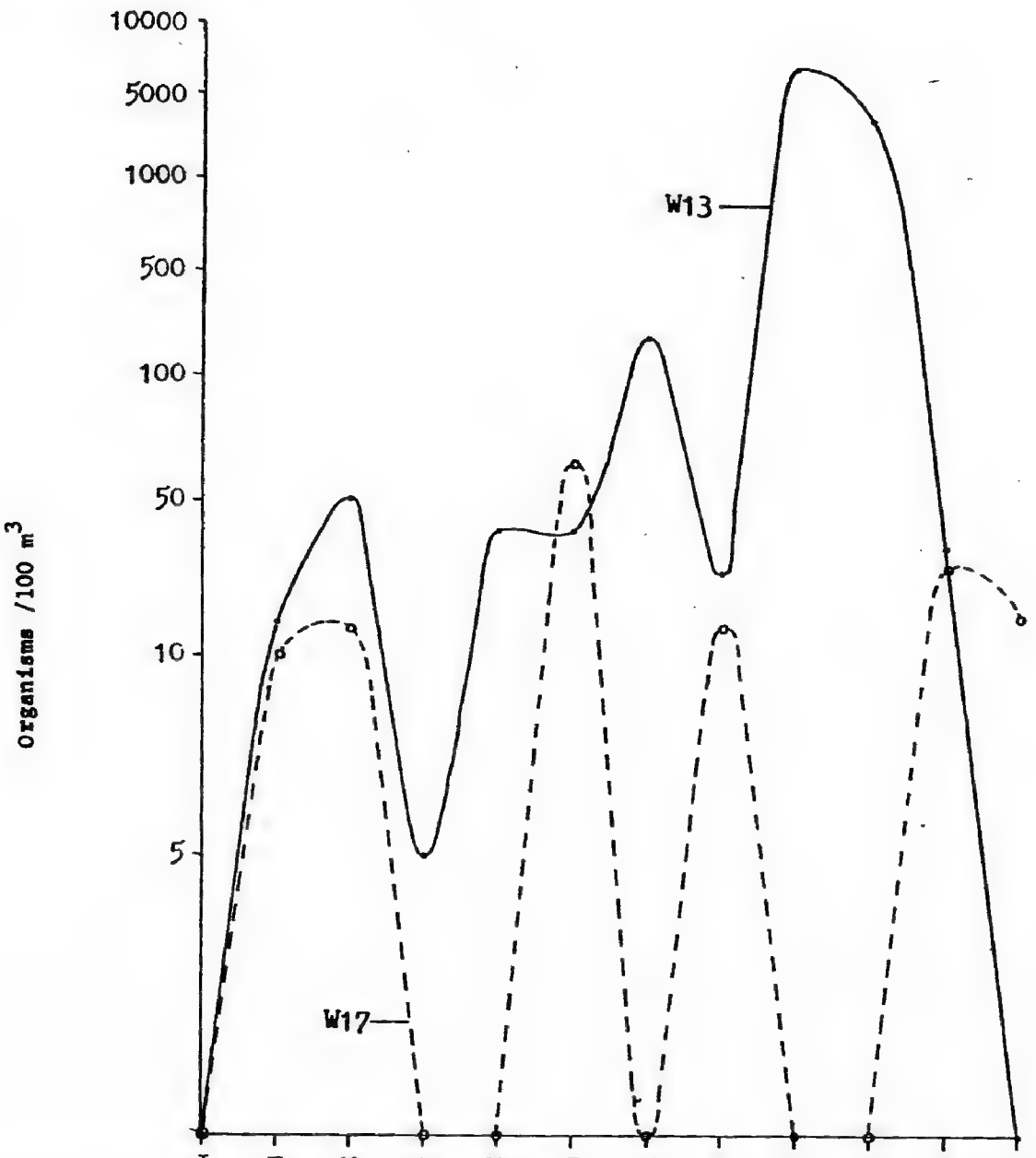

FIG. 6. Comparative seasonal distribution of *L. faxoni* at stations W13 (14 m) and W17 (81 m) plotted on a semi-log scale.

TABLE 1

Occurrence of adults, young and larval stages of *L. faxoni* in relation to three temperature and salinity ranges expressed as organisms/100 m³. Figures in parentheses indicate the percentage of total organisms collected during the entire year.

Temperature range (C)	Total occurrences	Adults	Young	Larvae	Total
11.8–19.0	10	45	63	49	157
		(0.2)	(0.2)	(0.2)	(0.6)
19.1–26.3	..	227	926	1,790	2,943
		(0.8)	(3.2)	(6.2)	(10.2)
26.4–30.0	..	3,246	3,056	19,478	25,790
		(11.2)	(10.6)	(67.4)	(89.2)
Salinity range (ppt)	**Total occurrences**	**Adults**	**Young**	**Larvae**	**Total**
26.1–32.8	11	108	163	5,098	5,369
		(0.4)	(0.6)	(17.6)	(18.6)
32.9–34.9	10	482	1,094	4,745	6,321
		(1.7)	(3.8)	(16.4)	(21.9)
35.0–37.3	11	2,928	2,788	11,474	17,190
		(10.1)	(9.7)	(39.7)	(59.5)

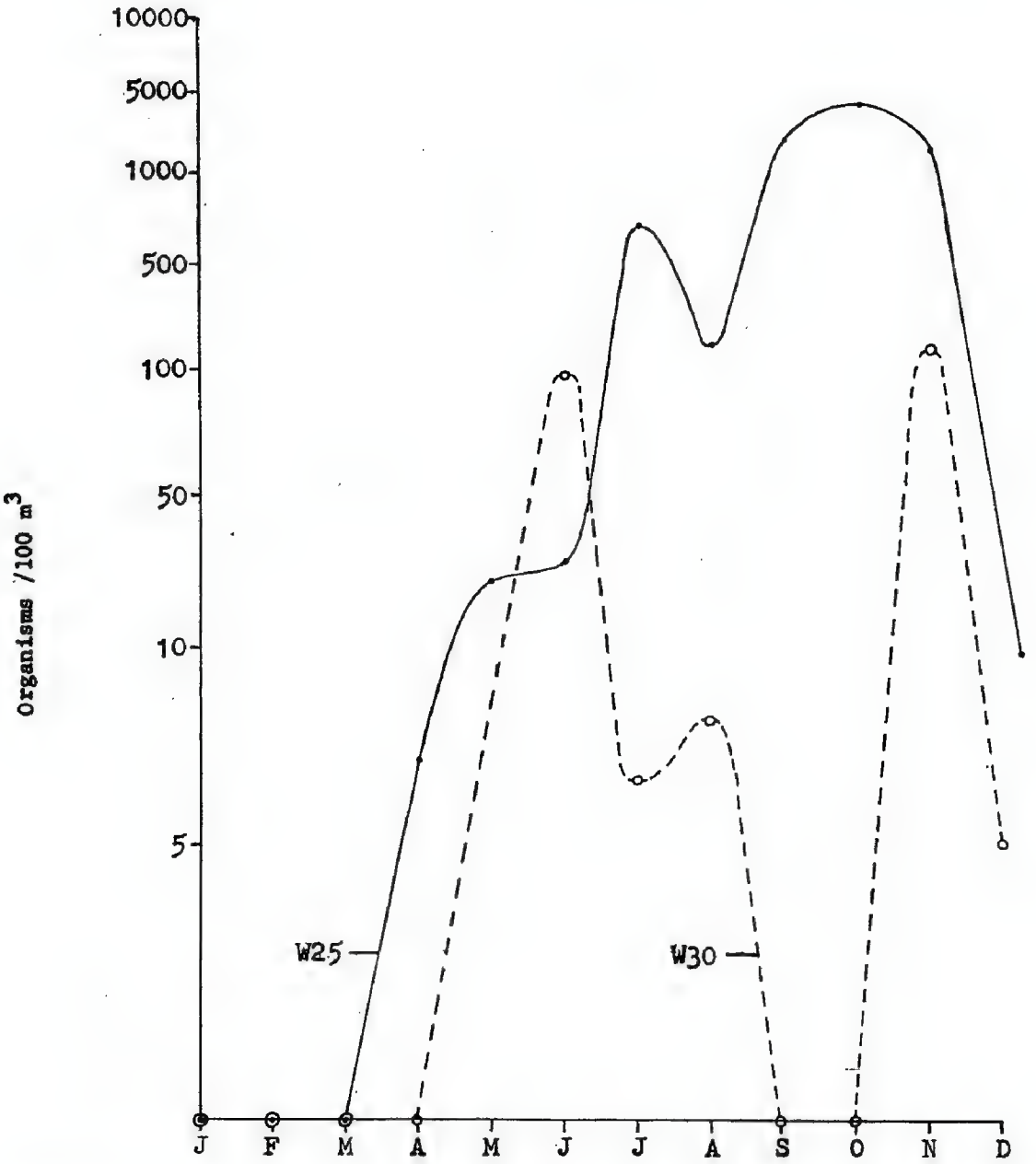

FIG. 7. Comparative seasonal distribution of *L. faxoni* at stations W25 (14 m) and W30 (108 m) plotted on a semi-log scale.

indicates that low ranges of both features were associated with low numbers of animals. Conversely, the largest numbers of organisms were associated with the highest temperature and salinity ranges. Similar results were obtained by Hopkins (1966) and Kelly and Dragovich (1967). The percentage of adults and young which occurred in the low temperature and salinity ranges were remarkably similar, as were the percentages in the middle and high temperature and salinity ranges. Low temperatures, however, appeared to be more restrictive to larvae than low salinities, and higher temperatures somewhat more conducive to larvae than high salinities.

While examining the animals, I noted that most specimens at W1 and W13 were sexually mature at a length of 8.0 mm, but at W25, they were sexually mature at a length of about 9.5 mm. Because the water warmed more slowly at W25 than at W1 or W13, it is possible that sexual maturation was retarded by the lower water temperatures.

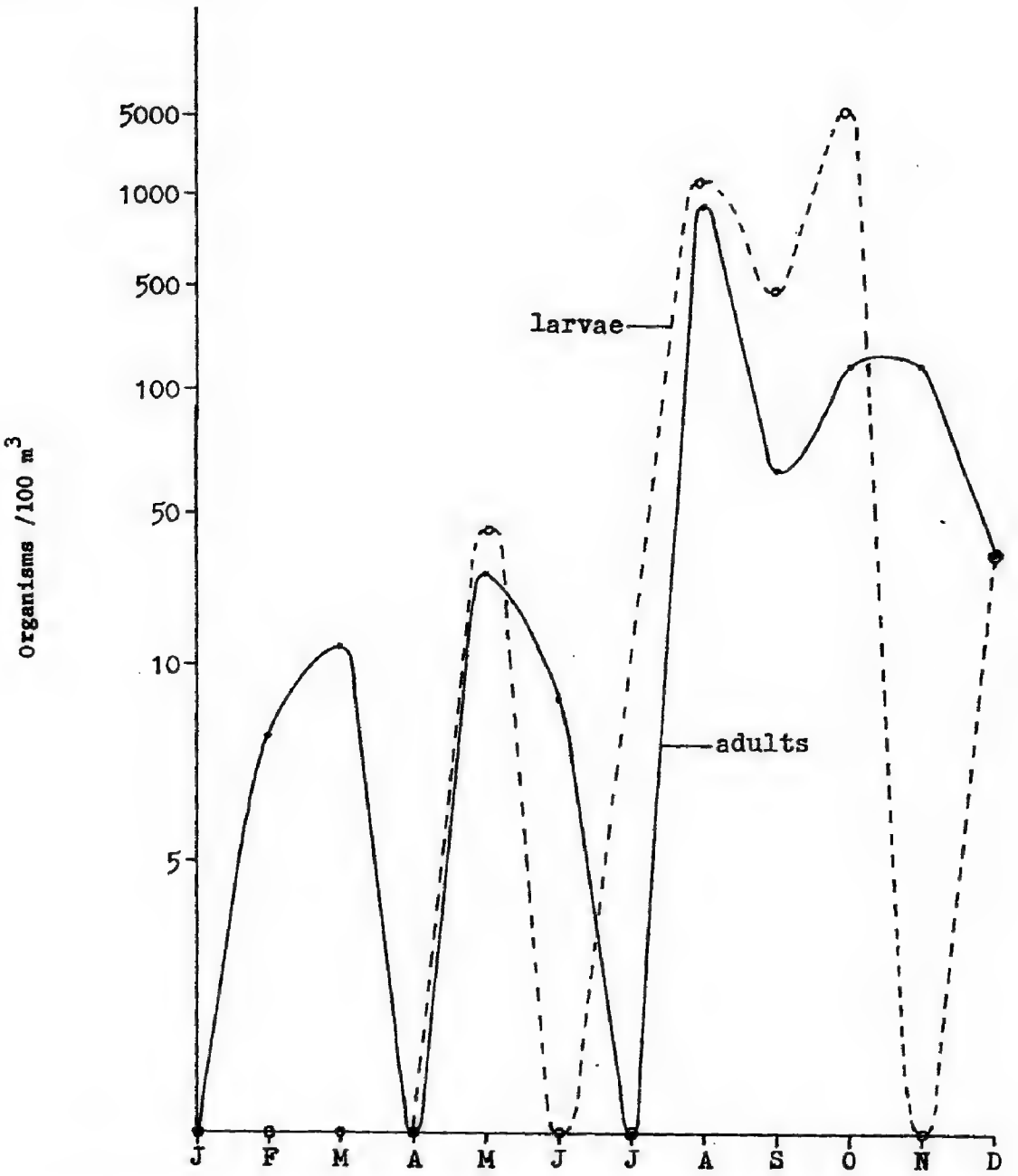

FIG. 8. Comparative seasonal distribution of *L. faxoni* adults and larvae at station W1.

POSSIBLE ENVIRONMENTAL FACTORS AFFECTING DISTRIBUTION OF *LUCIFER FAXONI*

In late summer and early fall of 1962, conditions at inshore (14 m) stations were optimal to produce large populations of *L. faxoni*. Because larvae constituted a major part of the fall population that was predominantly confined to nearshore waters, *L. faxoni* apparently has an autumnal, inshore peak reproductive period along the Texas coast. Although peak reproduction occurs within a relatively short period of time, the presence of sexually mature adults and the occasional presence of larvae throughout the year indicate that mating occurs continuously, although on a greatly diminished scale.

Water movements in the study area probably have a considerable effect on *Lucifer's* distributional pattern. Woodmansee (1966) found an apparent relationship between the daily vertical migration of *Lucifer faxoni* and solar and tidal cycles at two stations off Mississippi Sound. He suggested that the daily vertical migration of *Lucifer* was being modified to achieve landward transport by the

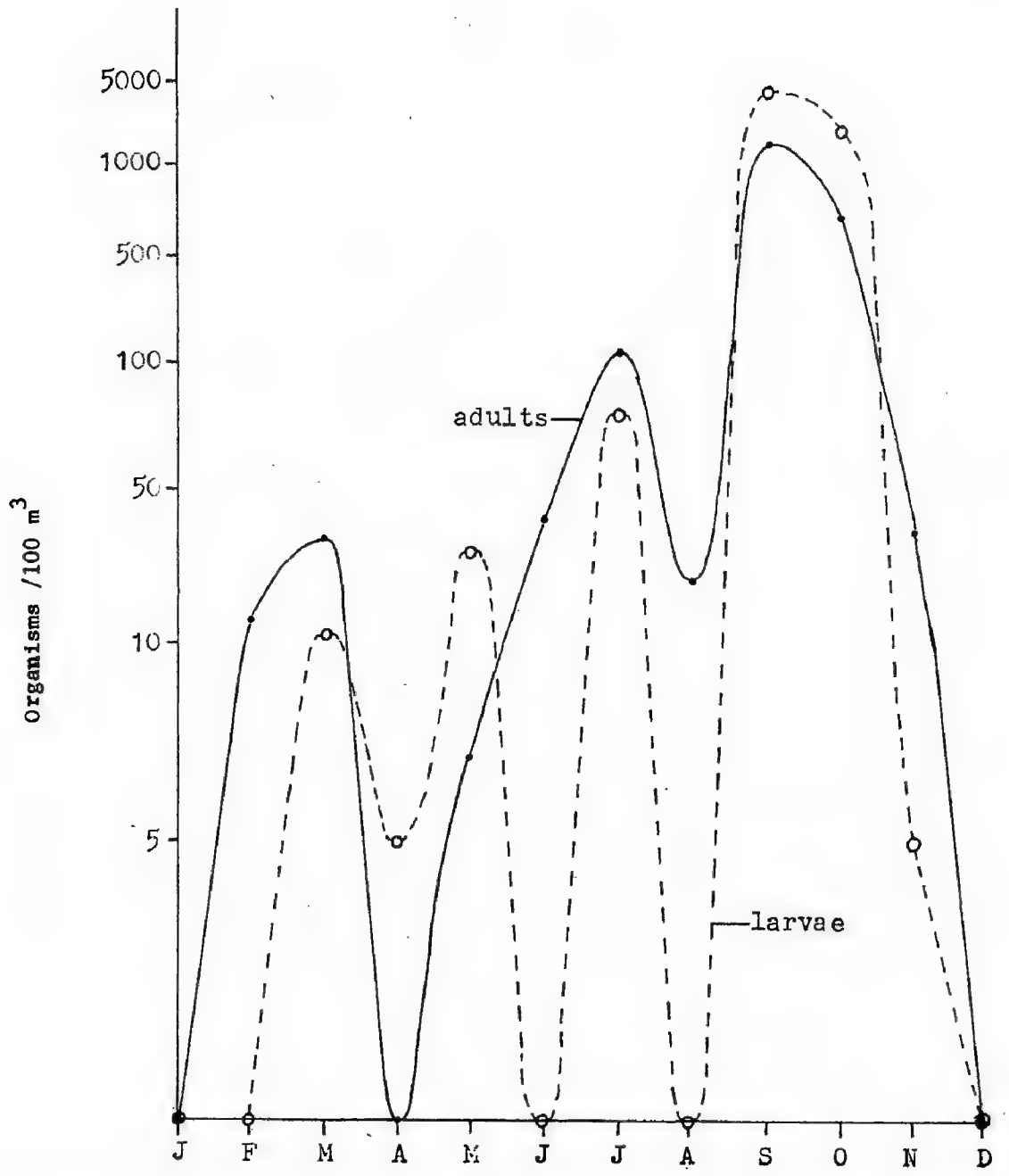

FIG. 9. Comparative seasonal distribution of *L. faxoni* adults and larvae at station W13.

tendency to swim into, or remain up in, the water column on flood tide and to drop out of the water column on ebb tide.

Currents along the Texas coast are onshore in May and June, becoming westerly and alongshore in July (Kimsey and Temple 1962). By September, currents shift to easterly and alongshore and remain so through December. *Lucifer* may achieve landward transport by its vertical migration cycle assisted by onshore water movements. The westerly, alongshore current may also partly explain successive westerly population increases.

As yet unexplained is the mode of population dispersal in late fall. A major factor may be a reversal of the vertical migration cycle demonstrated by Woodmansee (1966), the animals swimming up into ebb tide and dropping out of flood tide to achieve seaward transport.

Few data exist concerning factors which govern onset of horizontal migration among crustaceans. Bainbridge (1961) suggested that seasonal temperature changes might be effective, acting directly or through related physiological processes, or that physiological changes associated with growth may initiate on-

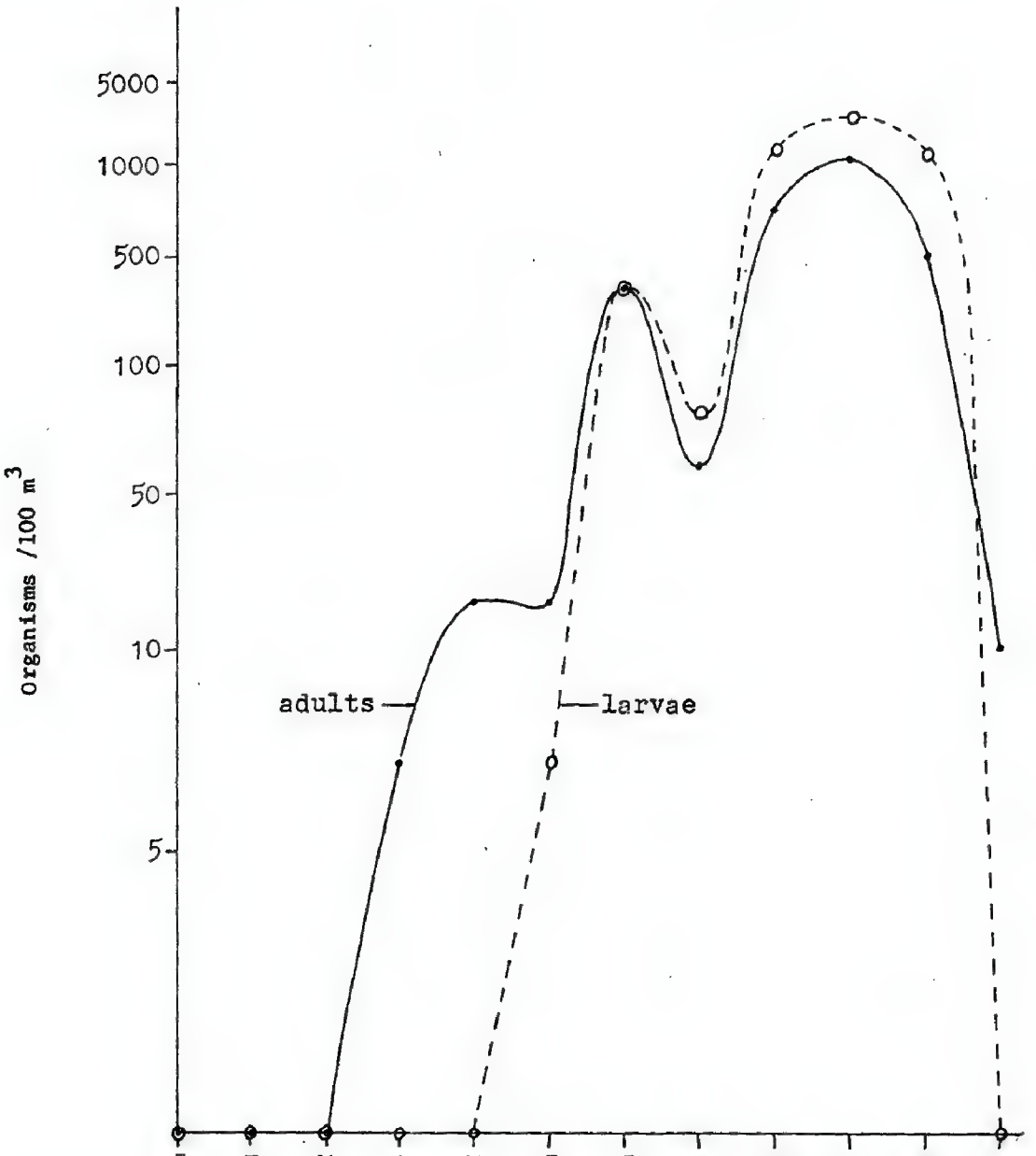

FIG. 10. Comparative seasonal distribution of *L. faxoni* adults and larvae at station W25.

shore migrations. Because highest inshore water temperatures were associated with large numbers of larvae, a relationship apparently exists between increasing water temperature and large inshore populations. Increasing water temperature may trigger the onset of horizontal migration as suggested by Bainbridge (1961). Assisted by water movements, the adults become concentrated in nearshore waters. This may greatly facilitate mating and result in the large larval population collected from August to November.

Lucifer nauplii probably rely on stored yolk material for nutrition as do other penaeid nauplii, but the succeeding larval stages are planktotrophic and must have a food source. At three stations in Alligator Harbor, Florida, Marshall (1956) measured chlorophyll *a* in plankton. His measurements indicated that the highest phytoplankton mass was in early summer followed by a second peak in the fall. Hopkins (1966) found that the biomass peaks of the eight principal diatom genera in St. Andrew Bay, Florida, were from July to November, concomitant with the increased *L. faxoni* population. In the surf zone off Mustang Island, Texas, McFarland (1963) reported that plankton productivity was lowest from fall to spring and highest in summer and early fall; but, because produc-

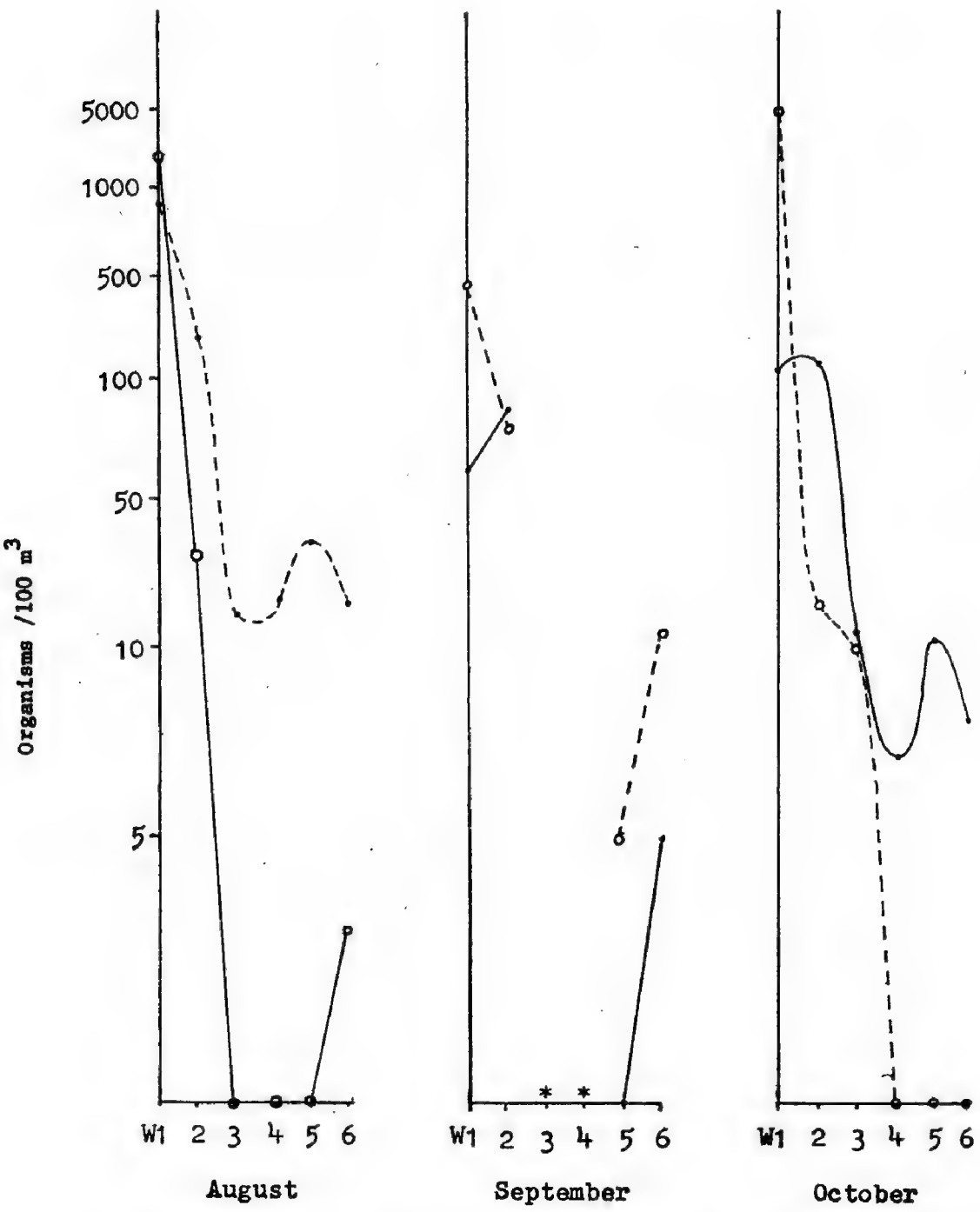

FIG. 11. Comparative bathymetric distribution of *L. faxoni* adults and larvae between stations W1 and W6 in the months of peak populations. *—denotes missing sample.

tion in overlapping months was quite different, he suggested that yearly variation was probably quite high. McFarland, however, noted high net photosynthesis in late summer and fall and suggested that phytoplankton constitutes a major source of organic matter for higher trophic levels. Thus, it appears that onshore migration of *Lucifer faxoni* adults may coincide with increasing water temperature and the autumnal phytoplankton bloom, both factors having great species survival value by providing optimum growth conditions during larval development.

NOTE ON THE OCCURRENCE OF *LUCIFER TYPUS*

Seventeen adult *Lucifer typus* H. Milne-Edwards (1837) were in offshore samples from W5, W6, W17 and W25. All occurrences, except one in March,

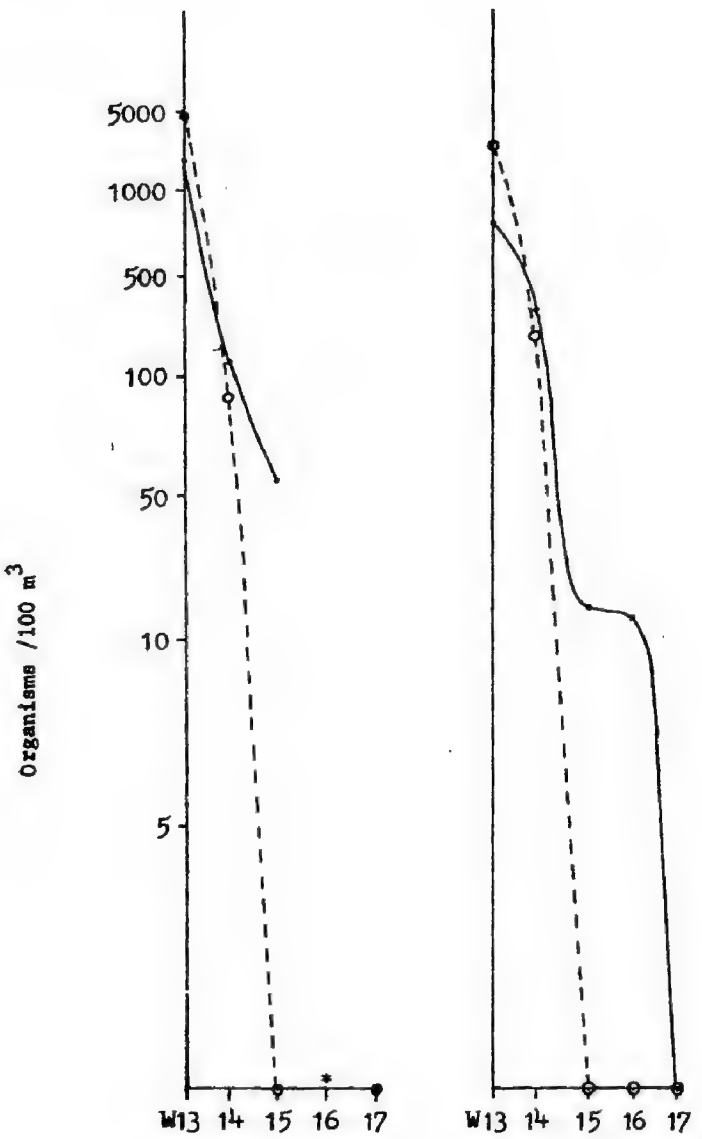

FIG. 12. Comparative bathymetric distribution of *L. faxoni* adults and larvae between stations W13 and W17 in the months of peak populations. *—denotes missing sample.

were between July and November, coinciding with the large inshore populations of *L. faxoni*.

L. typus is primarily an inhabitant of the oceanic province and does not usually occur inside the 200 meter depth contour according to Bowman and McCain (1967). It is probably carried occasionally into neritic waters by currents. As far as could be determined by a literature search, this is the first record of *Lucifer typus* in the Gulf of Mexico.

ACKNOWLEDGMENTS

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I wish to thank the members of my committee, Drs. J. G. Mackin, Sewell H. Hopkins and Sammy M. Ray, for their guidance during the preparation of the thesis. Plankton samples were made available for this study by Mr. Milton Lindner, Director, U.S. Fish and Wildlife Service Laboratory, Galveston, Texas. Mr. Robert F. Temple, head of the shrimp fisheries program of the Galveston Laboratory, aided in establishing the initial subsampling pattern and made numerous helpful suggestions during the preparation of the manuscript. Finally,

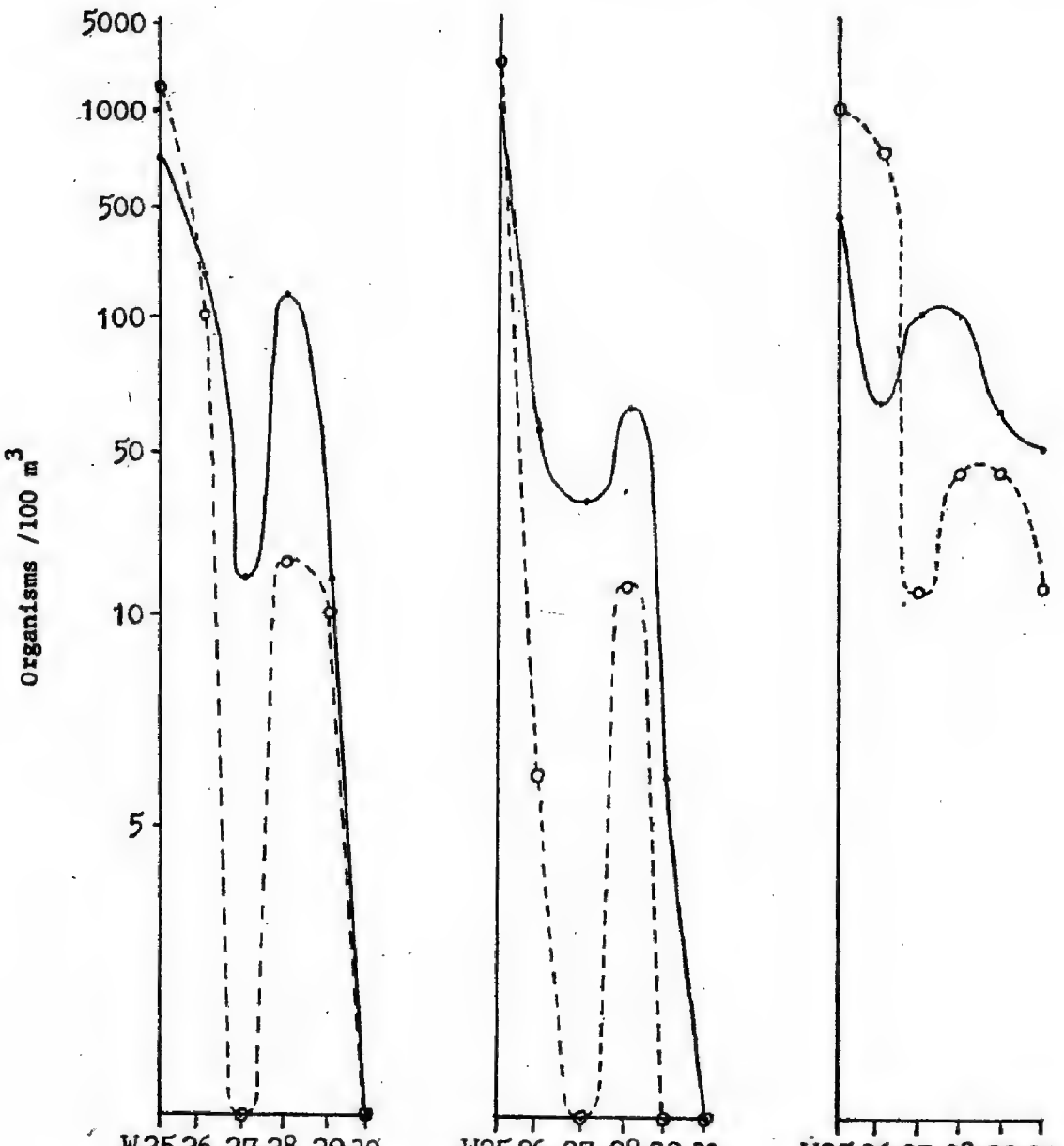

FIG. 13. Comparative bathymetric distribution of *L. faxoni* adults and larvae between stations W25 and W30 in the months of peak populations.

I wish to thank Dr. Harold W. Harry for critically reviewing this manuscript. This project was supported in part by Health, Education and Welfare grant No. EF–00621.

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USE OF DORSAL CARINAL SPINES TO DIFFERENTIATE BETWEEN POSTLARVAE OF BROWN SHRIMP, *PENAEUS AZTECUS* IVES, AND WHITE SHRIMP, *P. SETIFERUS* (LINNAEUS)[1]

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ABSTRACT

The presence of spines on the dorsal carina of the sixth abdominal segment of postlarval brown shrimp, *Penaeus aztecus* Ives, distinguished them with almost 100-percent reliability from postlarval white shrimp, *P. setiferus* (Linnaeus), in field collections from the tidal pass at Galveston, Texas.

INTRODUCTION

The presence of spines on the dorsal carina of the sixth abdominal segment of postlarval brown shrimp, *Penaeus aztecus* Ives, and pink shrimp, *P. duorarum* Burkenroad, and the absence of spines on the dorsal carina of postlarval white shrimp, *P. setiferus* (Linnaeus), were reported by Ringo and Zamora (MS). They concluded that this character was potentially useful in separating postlarvae of brown and pink (grooved) shrimp from white (nongrooved) shrimp. Their conclusions were based, however, on shrimp of known parentage reared in the laboratory.

[1] Contribution No. 266, Bureau of Commercial Fisheries Biological Laboratory, Galveston, Texas.

The objective of the present study was to determine the accuracy of the carinal spine character in separating postlarvae of brown and white shrimp taken in field collections.

METHODS

Postlarval shrimp were collected from the tidal pass at Galveston, Texas with a hand-drawn beam trawl and transferred alive to a holding tank in the laboratory. Each postlarva taken from the holding tank was placed in a depression plate, examined for the presence or absence of carinal spines, measured (total length—tip of rostrum to tip of telson), and placed in one of two tanks, depending on whether or not spines were present. No shrimp were discarded, and individual rearing tanks were used for each separated sample.

The postlarvae were fed brine shrimp (*Artemia* sp.) and reared to the juvenile stage ($\geq$ 25 mm total length) by a technique described by Zein-Eldin (1963). After the shrimp became juveniles, they were taken from the tanks, preserved in 10-percent buffered formalin, and identified to confirm the earlier species separation based on the presence or absence of carinal spines.

ACCURACY OF IDENTIFICATION BASED ON THE CARINAL SPINE CHARACTER

The numbers of postlarval shrimp separated on the basis of the presence or absence of carinal spines, sampling dates, and mean total length of shrimp in each separated sample are shown in Table 1. The same table gives the numbers of postlarval shrimp reared to the juvenile stage for final identification. All

TABLE 1

Number and mean length of postlarvae separated according to the presence or absence of dorsal carinal spines and the number of juveniles identified as brown or white shrimp in relation to the initial identification based on the spine character

	Postlarvae in sample and mean total length				Identification of juveniles in relation to initial postlarval identification			
	Spines present		Spines absent		Spines present		Spines absent	
Sampling date	Number	mm	Number	mm	Brown	White	Brown	White
1967								
Feb. 14	150	12.3	0	..	135	0	0	0
Feb. 28	161	12.0	0	..	150	0	0	0
Mar. 13	150	12.0	0	..	118	0	0	0
Mar. 27	140	12.2	0	..	112	0	0	0
Apr. 13	150	12.6	0	..	106	0	0	0
May 10	142	10.4	0	..	106	0	0	0
May 26	26	10.5	100	6.9	18	0	0	93
June 27	74	10.5	99	7.0	57	1	0	88
July 11	125	10.7	20	7.0	93	0	0	13
Aug. 16	100	10.0	150	7.0	65	0	1	89
Sept. 14	79	11.0	168	8.0	8	0	0	136
Oct. 16	22	12.0	49	10.0	11	1	0	43
Total	1,319		586		979	2	1	462

juveniles were identified by the characters given by Burkenroad (1939) and Williams (1953).

The use of the carinal spine gave almost 100 percent accuracy in separating postlarvae of brown shrimp from postlarvae of white shrimp (Table 1). Of 979 postlarvae that had spines and survived to become juveniles, all but 2 were brown shrimp. Of 462 postlarvae without spines that survived to the juvenile stage, all but 1 were white shrimp.

The carinal spine character has proven useful in separating postlarvae of brown and white shrimp in the Galveston Bay area and in Louisiana (Woodrow Mock, Louisiana Wild Life and Fisheries Commission, Grand Isle, Louisiana; and Curt Rose, Francis T. Nicholls State College, Thibodaux, Louisiana, personal communication). The character is especially useful in parts of Texas, Louisiana, and other locations in the northern Gulf of Mexico because pink shrimp are rare in these areas (Baxter and Renfro 1967; Kutkuhn 1962).

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CYCLIC AND CURRENT STRUCTURES IN A SERPULID REEF

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ABSTRACT

Reef-like serpulid worm growth in a power plant's concrete cooling water intake tunnels is cyclic, with a new generation apparently taking over each spring. The surface of the reefs is rippled, and tubes within the ripples are strongly current oriented.

Individual serpulid worm remains are known from the Ordovician to the Recent and reef-like colonies from the Cretaceous to the Recent. A knowledge of the ecology of living serpulids is, of course, essential to understanding the environmental conditions represented by their fossils. Although a little is known about the ecology of individuals of a few species (Hartman 1957), no work has been done on living reefs. This paper describes some cyclic and current related structures observed in a living reef and discusses their probable significance.

Cyclic structures in reefs of serpulid worm tubes have been described by Andrews (1964). Specimens of dead reefs from Laguna Madre and Baffin Bay, Texas show alternating layers in which the worm tubes first encrust a surface and grow sinuously and randomly on it (Fig. 1a) and then turn up from it and grow parallel to each other and more or less perpendicular to the surface (Fig. 1 b,c).

Similar cyclic growth has been found in reef-like masses of serpulids growing

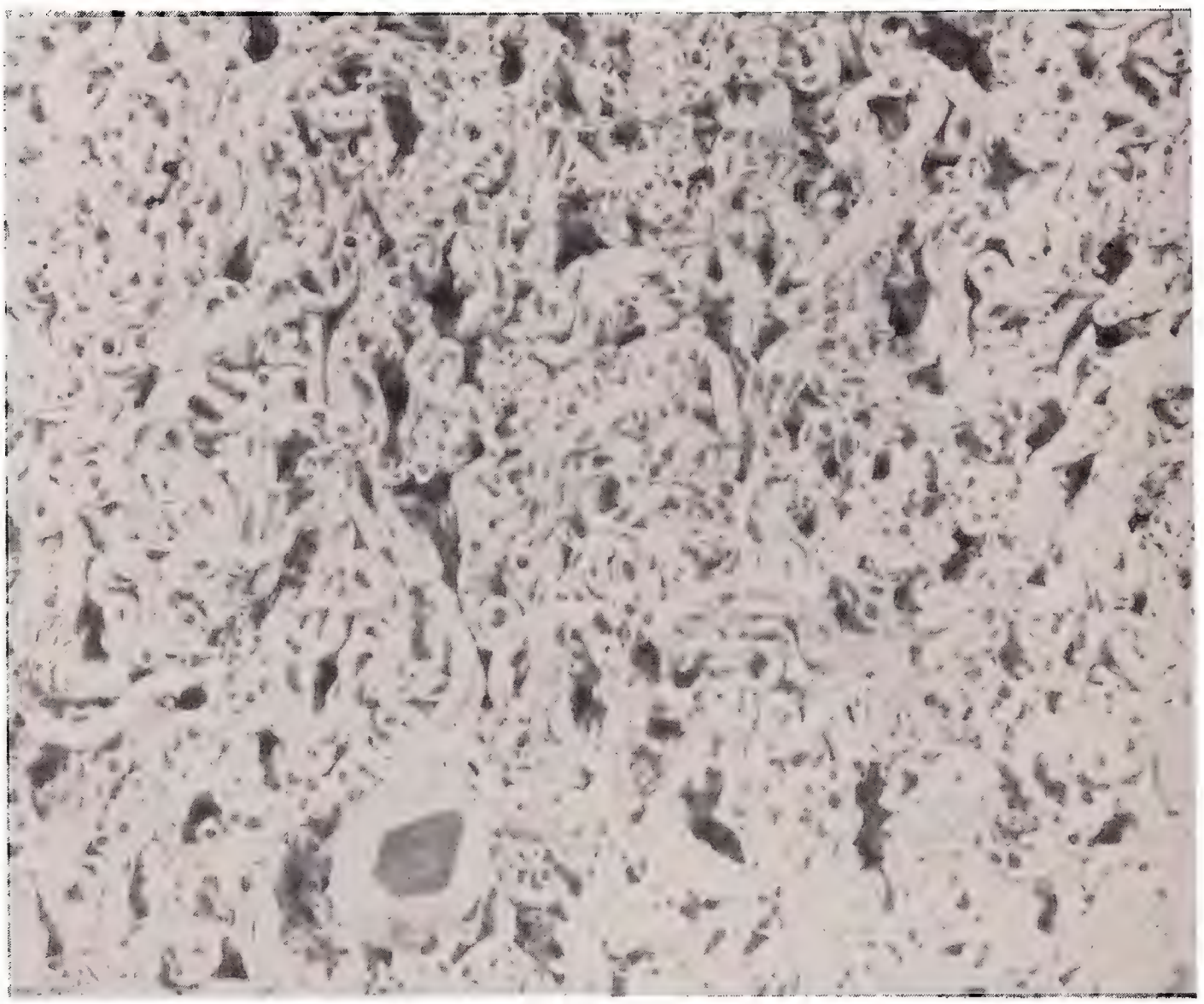

FIG. 1a. Serpulid worm tubes shortly after colonization of a freshly exposed surface. The tubes are more or less parallel to the surface but grow randomly with respect to each other. Barnacle shell is in lower center of the photograph. From a reef near the mouth of Baffin Bay, Texas.

FIG. 1b. Serpulid worm tubes growing parallel to each other and perpendicular to the surface of the reef. Same specimen as in Figure 1a.

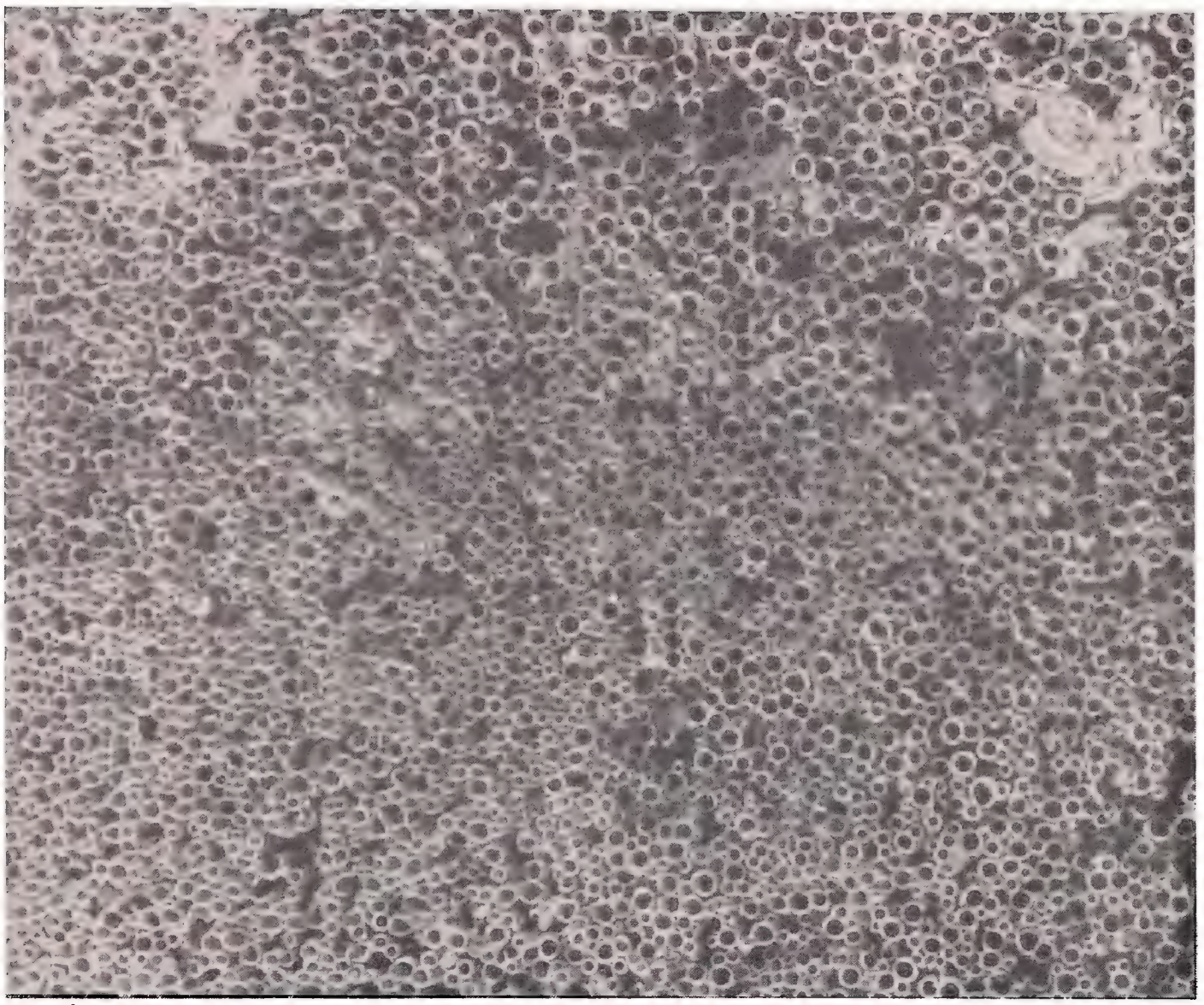

FIG. 1c. Section cut perpendicular to the face of the serpulid reef shown in Figure 1b.

in concrete tunnels at the harbor of Corpus Christi, Texas (Fig. 2). The tunnels are 6 × 8′ in cross section and about 500′ long (Fig. 3). Water taken from about 30′ below the surface of the harbor is drawn through them at the rate of about 130,000 gal/min. to cool electrical power generators and then is exhausted into Nueces Bay. Flow rates through various parts of the system range from one to three knots. These currents do not seem to prevent growth on any surfaces.

The serpulid worm forming the growths is *Hydroides norvegica* Gunnerus. It has been previously reported from the Gulf of Mexico only on the bottoms of ships frequenting Corpus Christi Bay (Hartman 1952). It is cosmopolitan, occurring from the Barents Sea above the Arctic Circle through the tropics to Australia. Like *Mercierella enigmatica* Fauvel and perhaps other adaptable, ubiquitous serpulids, this species may well have been distributed so widely by man through inter-oceanic shipping.

Growth in the tunnels restricts flow and must be periodically removed. The specimen shown in Figure 4 represents about 30 months of growth between the fall of 1964 and the spring of 1967. Three cycles can be discerned. Each begins with a large number of small tubes intertwining very irregularly more or less parallel to the surface of the colony. Then the tubes turn up from the surface and grow away from it. Thus, with respect to gross orientation, the growth is the same as in natural reefs.

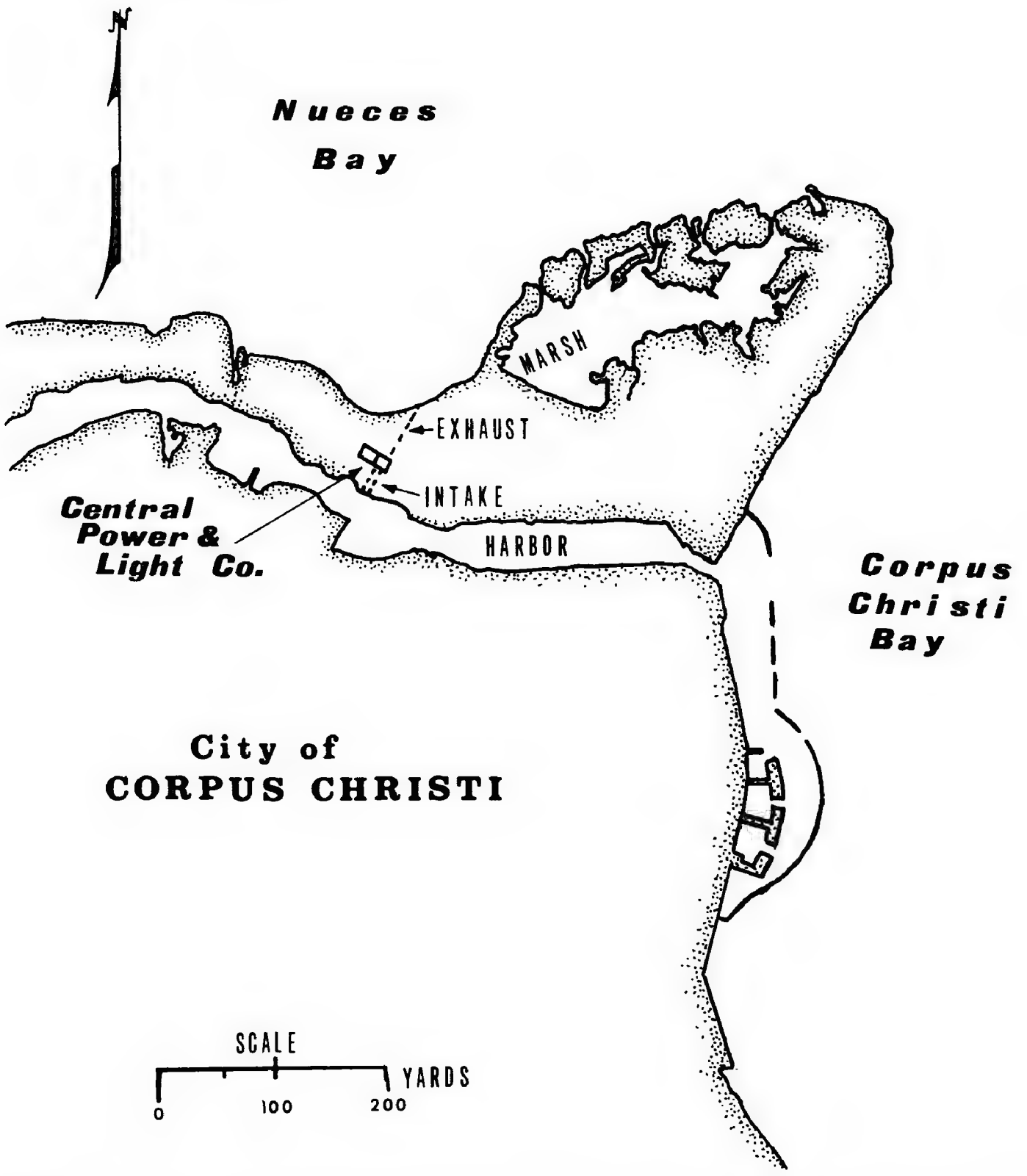

FIG. 2. Location map showing the cooling water intake tunnels on the North side of the harbor of the city of Corpus Christi, Texas.

Larval setting at Port Aransas suggests that heavy serpulid spawning begins in April (Fig. 5). The large number of small tubes at the base of each cycle probably represent new generations setting first on a clean surface during the fall of 1964, then on pre-existing colonies in the springs of 1965 and 1966. The 1967 generation was not yet established when the block was removed.

Growth rates for the first month are higher than those at subsequent times (Edmondson and Ingram 1939). Growth rates of tubes growing on the sides of aquaria have been measured from 1.5 to 2.5 mm/day for the first three days of growth; but these rates decrease to from 0.8 to 1.3 mm/day for the next two to

FIG. 3. Interior view of intake tunnel with 30 months of serpulid growth. Ripples are on all walls but are visible only on the ceiling and left wall due to the lighting.

four weeks. The rates then decrease further to from 0.1 to 0.2 mm/day. Thus it is not surprising that few if any worm tubes extend from one cycle into the next, because the young worms crowd out the old by simply growing 10 to 20 times faster.

Although a few measurements suggest that growth rates are higher in the strong current situation in the cooling water intake tunnels, rates within the ranges measured in aquaria can easily account for the thickness of growth during the 1964—1967 period. For example, the middle cycle represents 12 months and is 85 mm thick. If one assumes a rate of 1.0 mm/day for the first 30 days, then 55 mm of this cycle must have grown in about 330 days or at a rate of 1/6 (0.167) mm/day. Likewise if 90 mm of the total specimen thickness of 200 mm is accounted for by three separate months of initial growth rates of 1.0 mm/day, then the remaining 110 mm must have grown at a rate of 0.136 mm/day.

In addition to the gross orientation already described, the serpulid worm tubes in the cooling water intake tunnels form distinct current related structures. The surfaces of the colonies appear ripple marked (Figs. 3, 4) with wave lengths from 10 to 15 cm, wave heights from 1 to 2 cm, and ripple crest lengths from 10 to 20 cm. The structures are strongly asymmetric with the gentle slope up current

FIG. 4. Block of reef removed from the tunnel shown in Figure 3. Current was from right to left. Note the dense packing of tubes on the ripple surface in the center of the upper surface and in the center of the middle cycle, and the looser packing to either side.

and the steep slope down current, much the same as current ripples in sand or other loose sediment.

The current would be fastest on the gently rising up-stream side of the 'ripple.' Here the tubes are tightly packed and oriented subparallel to the surface with the tube opening pointing down-stream. In the lee of the 'ripple' crest an eddy probably slows the current and gives it a very irregular path. The tubes here are much more loosely packed and grow irregularly more or less perpendicular to the surface. The worms seem to grow, then, simply in the direction the current bends the soft bodies when they are partially extended from their tubes. So far as is known, no such current structures have been reported from other serpulid reefs.

ACKNOWLEDGMENTS

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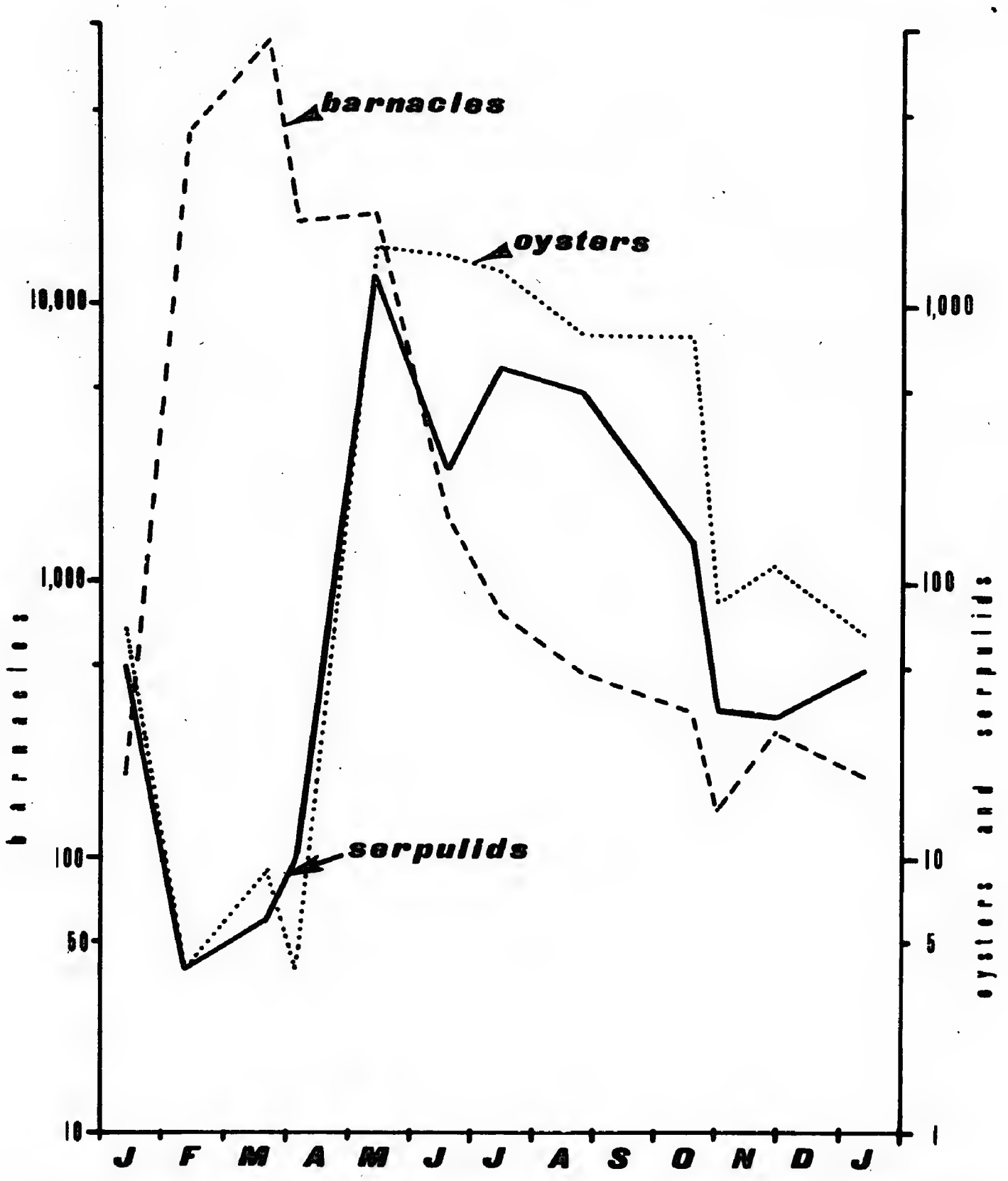

FIG. 5. Numbers of larvae of the three most abundant fouling organisms settling and metamorphosing on filter screens in flowing sea water system at the University of Texas Marine Science Pier Laboratory on Aransas Pass inlet, Port Aransas, Texas.

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A STUDY OF THE GEOCHEMISTRY VITAMIN B_{12}

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ABSTRACT

Vitamin B_{12} has been quantitatively measured in recent sediments taken from several environments along the Texas coast. The upper few cm of sediment is rich in B_{12}, but it falls below the limit of detection in approximately one meter. Interstitial waters were found to be highly enriched with regard to the sea water above the sediment. It is suggested that microorganisms living in the sediment are supplying B_{12} to the overlying waters via the interstitial waters.

INTRODUCTION

Organic compounds in sedimentary formations of all ages are generally considered to be of biological origin. Typically, they constitute several percent of the sediment weight, although some sediments with as high as 30% organic carbon have been reported. The biological material laid down at the time of formation of a recent sediment is usually reworked and added to by biological action for a short time after deposition. These biochemicals are greatly altered and degraded by the subsequent chemical and physical processes which take place in geological time. Almost all of the original biological material is highly modified, so that only a small fraction of the organic matter in an ancient sediment has retained its biochemical identity. Most of it is incorporated into kerogen,

an insoluble polymeric substance of variable composition and unknown structure formed from the original organic compounds in the sediments.

However, traces of some of the original chemicals persist and can be characterized. Fatty acids, hydrocarbons, amino acids, steroids, terpenes (isoprenoid hydrocarbons), carbohydrates, purines, pyrimidines, porphyrins and other pigments all have been isolated from sediments of various ages (Breger 1963). The occurrence of many of these compounds in sediments supports a biological origin for all sedimentary organic material.

The vitamins, a group of compounds of unmistakably biological origin, have received very little geochemical study. Since they are trace compounds in organisms, very low concentrations would be expected in sediments. In recent years, dependable microbiological assays have been developed for most vitamins (Hutner *et al.* 1958). Many of these are very sensitive and specific, usually requiring little preparation of the sample. Few of them require even rough isolation of the compound of interest.

Vitamin B_{12} is a cobalt porphyrin with a slightly modified nucleus—two of the pyrrole rings are joined directly by a carbon to carbon bond instead of by a methine bridge (Smith 1960). Porphyrins are geochemically rather stable, but it is the tetrapyrrole nucleus that persists longest. The stability of the rest of the B_{12} molecule for geologic time periods was not known at the outset of this investigation.

Bacteria and fungi are the main and possibly only producers of B_{12} although it is both an animal and microbial growth factor. Since bacteria and fungi are abundant in surface sediments, they could serve as a source of B_{12} to the overlying waters. Vitamin B_{12} has been found in the uppermost few centimeters of recent muds by several workers (Burkholder and Burkholder 1956a, 1956b; Letunova 1958, 1959a, 1959b; Starr 1956) as well as in natural waters (Cowey 1956; Daisley 1958). These factors suggested that it would be worthwhile to look for B_{12} in geologically older materials.

The purpose of this work, then, was to see how far back in geologic time vitamin B_{12} could be detected. If it proved to be very stable it might serve as a biological marker in ancient sediments. Secondary goals were to determine the relation between B_{12} concentration and the amounts of organic carbon associated with it, to compare the amounts of B_{12} deposited in various environments, and to evaluate sediments as the probable source of the B_{12} in sea water.

METHODS AND MATERIALS

Sediment Samples

Samples were collected from several Texas bays and the Gulf of Mexico. The locations of the sampling stations are shown in Figure 1.

Short cores were taken in lengths of 1¼ inch diameter, stiff plastic tubing. Some were extruded in the field and wrapped in aluminum foil while others

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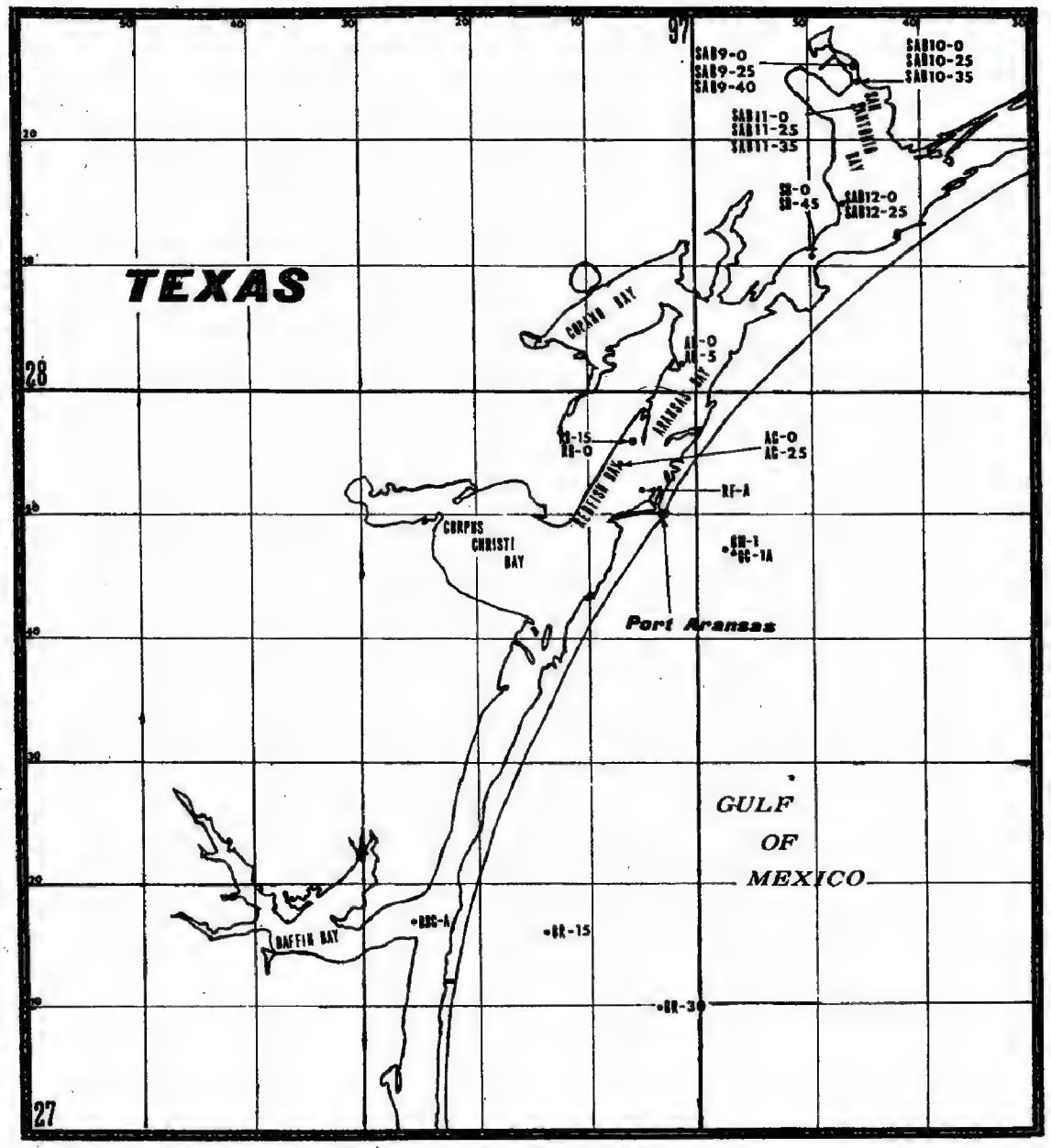
FIG. 1. Location of sample stations.
a: Coastal Bend Area.
b: Freeport Area.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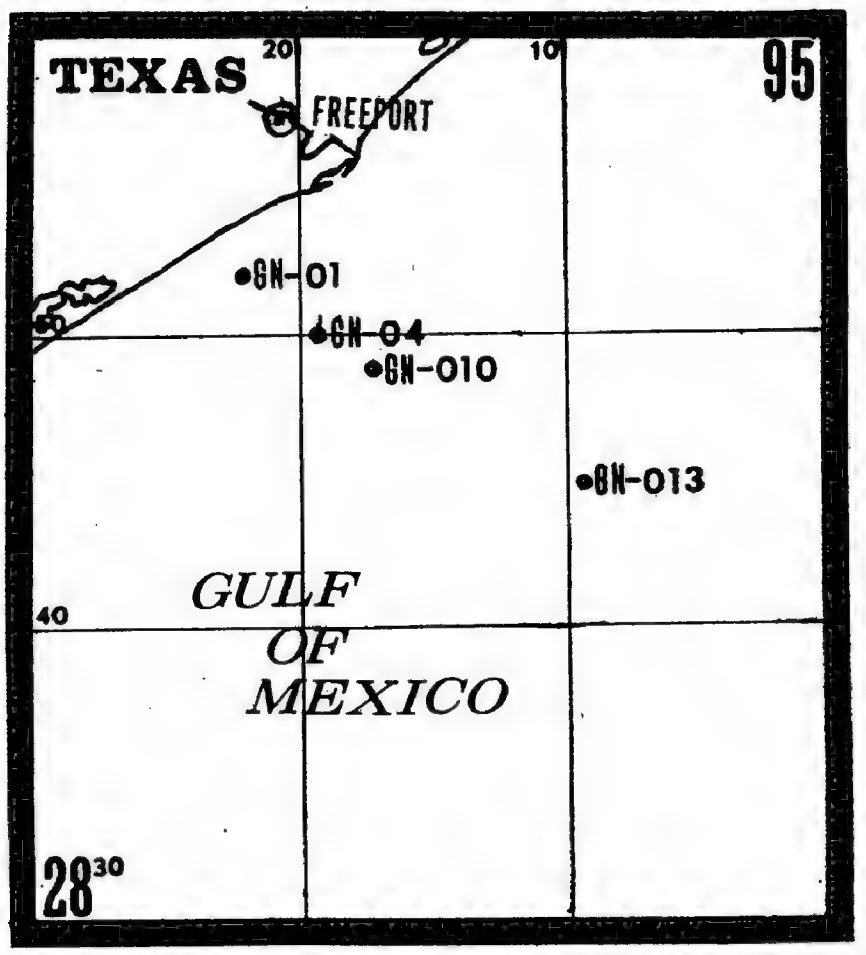
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were left in the tubes and extruded on return to the laboratory. They were all refrigerated immediately after collection. The longer cores were taken in three inch aluminum irrigation pipe fitted with a piston, from a barge constructed for the coring operation. These were extruded immediately, wrapped in aluminum foil and refrigerated as soon as possible. Gulf of Mexico cores (GC1, GC2, GC3) were taken with a trigger-type Ewing corer in 21 m of water about 10 miles southeast of Port Aransas, Texas. All cores were sectioned and sampled on return to the laboratory and frozen until used.

Grab samples from the Gulf of Mexico were taken at sites near the cores (GM0, GM1, GM2) and along two southeasterly transects, one off the the coast of Padre Island opposite Baffin Bay (GR), and the other from the mouth of the Brazos River near Freeport, Texas (GN). These were refrigerated immediately after collection.

The San Nicholas (SN) and the San Pedro (SP) grab samples were taken off the coast of California by Dr. T. C. Hoering (Carnegie Institution, Washington, D.C.). They were immediately refrigerated or frozen as soon as possible after collection.

The Antarctic core was taken in 16,500 feet of water from the south Atlantic by the *USNS Eltanin* and was obtained through the generosity of Florida State University.

Other samples

The fossil turkey dung and human feces were collected by the Wetherill Mesa Archeological Project from site 1229 in Mesa Verde National Park, Colorado, and were provided through the kindness of Dr. Douglas Osborne, Supervising Archeologist. They are estimated to be several hundred years old.

Crude petroleum samples were provided by Dr. Ellis E. Bray of Socony-Mobil, Dallas:

1. Sulfur Bluff Field, Hopkins County, Texas, Paluxy formation (Cretaceous), 4500 ft.
2. Quesera Field, West County, Texas (Eocene), 1128 ft.
3. Black Thunder Field, Weston County, Wyoming, Newcastle formation (Lower Cretaceous), 6883 ft.

The Green River Shale was collected by Dr. T. C. Hoering, of the Carnegie Institution of Washington, near Rifle, Colorado.

Butter was once preserved in Ireland by sealing it in wooden tubs and burying it in bogs. Misplaced specimens of this somewhat fossilized "bog butter" are occasionally unearthed, and two samples of it were obtained from the Irish National Museum. One is from Carnquill, County Galaway and the other from Shannakeela, County Monaghan. Both were determined to be around 400 years old by carbon-14 dating.

The worm feces were gathered from serpulid worm colonies being maintained at the University of Texas Marine Science Institute by Dr. E. William Behrens.

Extraction Reagent

All chemicals were reagent grade except where noted otherwise. All water

used was distilled from a glass still and stored in polyethylene dispenser bottles.

Buffers are generally used to extract water-soluble biological compounds for assay. Citrate-phosphate buffer of pH 5.0 was prepared by the method of T. C. McIlvane as outlined in *Methods of Enzymology, Volume I* (Gomori 1962).

Some investigators have found that when certain anions are added to the buffer, the yield of Vitamin B_{12} in extracts is increased (Burkholder and Burkholder 1956a, 1956b). Some of these were tried to determine their effectiveness for this work. By extracting identical samples of several muds with the buffer containing different enhancing agents and then assaying the extracts, it was found that the response of some samples was increased more by cyanide and others more by bisulfite. A mixture of the two in the buffer, 0.1% sodium bisulfite and 0.005% potassium cyanide (hereafter called the "enhanced buffer"), was selected as giving the best combination of the two effects. Cyanide increased the response of the standards as well as that of some samples. Bisulfite increased the yield of vitamin B_{12} in the samples examined by an average of 100%, not quite the increase (370%) observed by Burkholder and Burkholder (1956a). The bisulfite did not affect the response of the standards. Larger concentrations of cyanide than mentioned above are toxic to the assay organism.

Nitrite and pyrosulfite were also tried as enhancers but showed no noticeable effects on the B_{12} yield of the samples.

B_{12} Extraction

About 5 g of wet sample was weighed and placed into a test tube containing 5.0 ml of enhanced buffer. This was thoroughly mixed by agitating with a Vortex, Jr. mixer, heated for 20 minutes at 90 C in a water bath and then mixed again while still hot. The sample was centrifuged immediately and the extract decanted and used for assay.

The Green River Shale sample required special treatment. A piece of the shale was boiled for several minutes in distilled water, rinsed with the citrate-phosphate buffer to remove surface contamination and then crushed with a clean hammer, mortar and pestle to pass a NBS 20 sieve. A blank run with the buffer rinsings used to clean the tools showed they contained no B_{12}. The sample was then treated with HCl to remove carbonates and further break it up. The residue was removed by filtration. The filtrate and washings were diluted to known volume and assayed.

The stability of B_{12} to the extraction procedure was checked by heating 5 ml portions of 10 μg/1 standard for varying periods of time in a 90 C water bath, and then assaying them. No detectable loss of B_{12} was found in up to three hours heating at this temperature.

Standards

A stock of standard vitamin B_{12} was prepared from crystalline cyanocobalamin (U.S.P.—Sigma Chemical Company). Approximately 10 mg was weighed and dissolved in 100 ml of buffer with 0.005% KCN. Four ml aliquots of this stock were kept frozen in screw cap vials until diluted for use.

To prepare standards, 1.0 ml of stock was diluted to make quantitative dilu-

tions of about 100, 50, 25, 1.0, 0.5, and 0.25 micrograms per liter. The 0.25 μg/l concentration gave no measurable response to the assay and represents the lower limit of detection. None of the extracts assayed were above the 100 μg/l range.

Blanks

Beach sand was fired in a muffle furnace at 800 C to burn off all organic matter and then treated as other samples. Distilled water, tap water, buffer and enhanced buffer were also assayed for possible sources of contamination. Vitamin B_{12} was never detected.

Assay

Extracts were assayed by the filter paper disc (FPD) method (Hoff-Jorgensen 1957). The blue-green alga *Coccochloris elebans* strain 17a, hereafter called 17a (Van Baalen 1961), was used as the assay organism. *Coccochloris elebans* (17a) has an absolute requirement for vitamin B_{12} and little growth response to other compounds (Van Baalen 1961). Other types of B_{12} assays are available (Hoff-Jorgensen 1957) but the FPD method was chosen mainly for its simplicity. It requires none of the individual tube preparation and special equipment of the liquid culture assay. Furthermore, it usually takes less time and handling than other methods and can accommodate many samples at once.

The medium used was the ASP-2 medium of Provasoli, McLaughlin and Droop (1957) as modified by Van Baalen (1961).

Three types of cultures were used in the assay procedure:

Stocks; 17a was maintained on slants of ASP-2 plus 2 μg/l cyanocobalamin with 1% Difco Bacto-Agar and kept at room temperature under fluorescent lights (20 watt Cool White).

Liquid cultures; 17a was grown by liquid culture after the method of Myers (1950) in 20 ml ASP-2 with 2 μg/l cyanocobalamin in 39 C water baths illuminated by two 30 watt Deluxe Cool White lamps 7 cm from the tube centers on each side of the bath. One percent CO_2 in air was bubbled through the tubes, and every two days a drop of culture was back-transferred to a fresh tube of medium. Every two to three weeks the liquid cultures were renewed from the slants.

Depleted inoculum for assay; 20 ml of ASP-2 without cyanocobalamin was inoculated with 0.5 ml of the liquid culture. This was grown for three days under the same conditions as the liquid cultures before use.

The plates (Pyrex baking dishes, 25 cm × 35 cm), filter paper discs (12.7 mm S&S No. 740-E), ASP-2 with 1% agar (300 ml per plate) were sterilized by autoclaving for 20 minutes at 121 C. The agar medium was cooled to 40–45 C and seeded with 6 ml of the depleted inoculum for each plate and poured into the plates to a depth of about 4 mm.

The amount of depleted inoculum used and the depth of the medium in the plates was not critical since a set of standards was run on each plate. Variation among standards on plates of the same batch was never more than 6% and among the standards on all plates, never more than 10%.

Aliquots of samples and standards absorbed onto filter pad discs were placed on the seeded plates. The pads are designed to hold almost identical volumes when saturated (0.1 ml). Whenever possible, triplicate extracts were prepared from each sample and, in all cases, each extract was assayed in triplicate. Pads were spaced about 4 cm apart giving five rows of nine pads each. After a plate was filled, it was covered with Saran Wrap and placed in an incubator. After about an hour the plates were inverted to prevent condensation from dripping onto the pads.

Plate incubators were racks of six closely mounted (3 mm apart) fluorescent bulbs (30 watt Deluxe Cool White) 50 cm above the plates. The plates were protected from drafts and, with the help of a shielded 100 watt incandescent lamp, their temperatures kept between 28 and 32 C. Temperatures much lower or higher caused slower development of the growth zones.

After 3–4 days incubation, growth zones on the plates became apparent. Some were immediately measurable while others were set out at room temperature and light until the green color of the growth zones deepened enough to permit measurement with vernier calipers. Some of the plates were measured every other day for two weeks after removal from the incubator to determine any change in growth zone size with time. Zones remained essentially unchanged in size during this time.

Zone diameters of the standards were plotted against the logarithm of their B_{12} concentration, giving fairly straight lines with some deviation at higher concentrations (Fig. 2). Since most samples were in the lower range, this deviation was not a problem. The B_{12} concentrations in the extracts were found from these standard curves.

Many samples, although showing no measurable growth zone, had distinct green coloration beneath the pads indicating trace amounts of B_{12}. The B_{12} concentrations in these samples were estimated to be between the standard of no response and the lowest of noticeable response (0.1 and 0.25 μg/1, respectively).

RESULTS AND DISCUSSION

The concentrations of vitamin B_{12} found in recent sediments are of the order of magnitude of 10^{-9} grams of B_{12} per gram of dry sediment (Tables 1, 2 and 3). This represents one of the lowest concentrations of biochemicals that has been detected in recent sediments. Sediments similar to those of this work contain microgram quantities of carotenoids, sterols and fatty acids in each gram of sediment (Parker 1967).

Sediments from open water (Gulf of Mexico, San Nicholas and San Pedro Basins) generally have a higher B_{12} content than estuarine deposits (Tables 1 and 2). An interesting variation in the Gulf is the GN-01 and GN-04 samples. These contain markedly less B_{12} than the other Gulf sediments. However, both of these were taken from stations close to shore directly off the mouth of the Brazos River near Freeport, Texas. GN-010 and GN-013 are from deeper waters along the same transect and away from the river's influence. The higher Gulf values probably reflect better preservation rather than higher production of B_{12}.

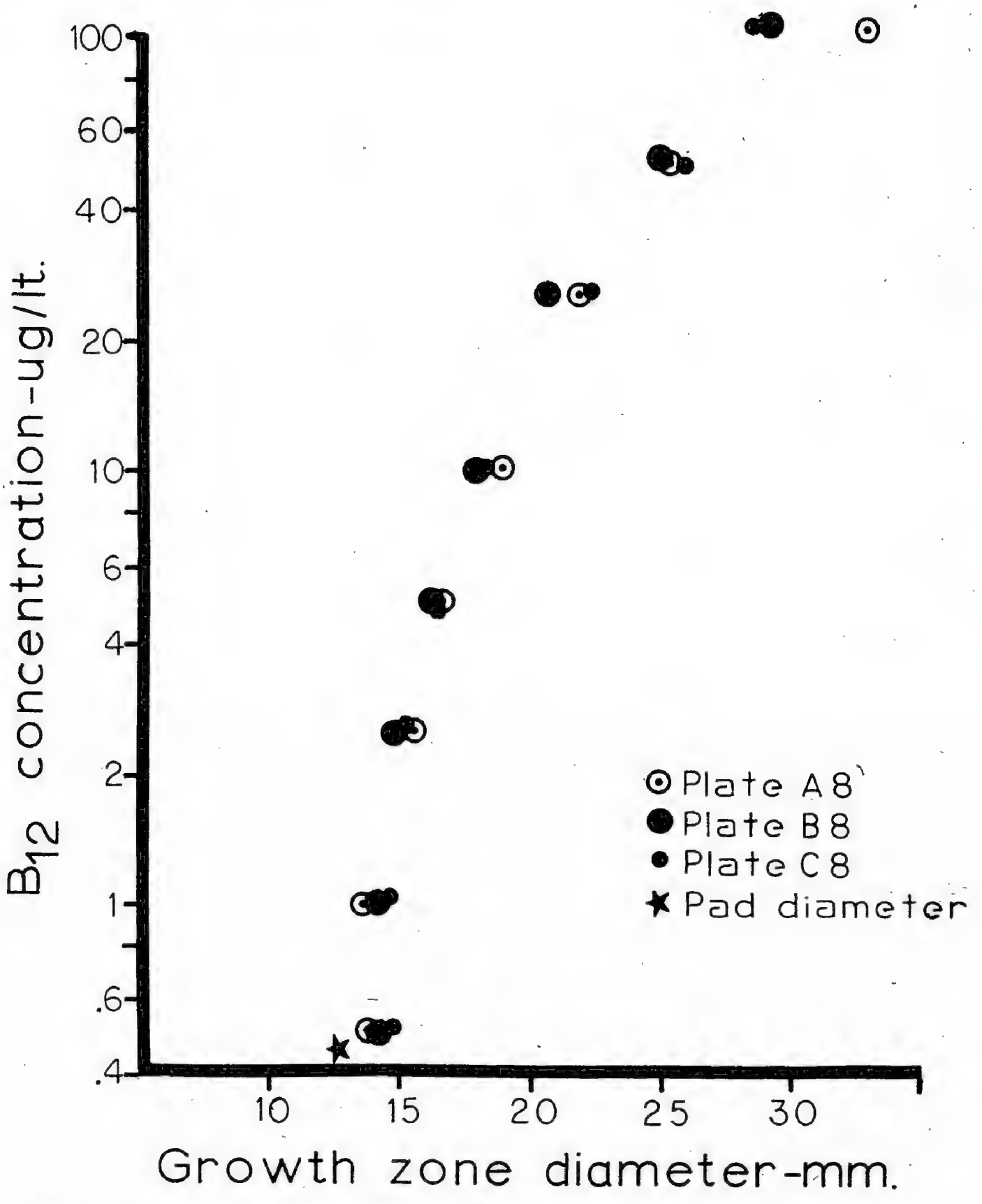

FIG. 2. Typical standard curves for vitamin B_{12} assay.

Estuarine sediments are stirred more by wind and waves than are the deeper Gulf sediments, and thus B_{12} could be lost to the water column.

Burkholder and Burkholder (1956b) and Starr (1956) have found that sediments and suspended solids in the overlying waves contain similar amounts of B_{12}. This is not unexpected since the suspended matter is the main source of the sediment. It is conceivable, though, that some sediments could be enriched in B_{12} from bacterial action aided by reworking after deposition.

The amounts of B_{12} reported by the above authors are considerably higher than

TABLE 1

Collection data and vitamin B_{12} contents of grab samples

Sample	Latitude	Longitude	Water depth (m)	B_{12} Content ng/g
Gulf of Mexico				
GR–15	27°16′N	97°14′W	26	25.0
GR–30	27°10′N	97°04′W	49	15.0
GN–01	28°52′N	95°22′W	3.5	4.2
GN–04	28°50′N	95°19′W	9	9.1
GN–010	28°49′N	95°17′W	19	38.0
GN–013	28°45′N	95°04′W	23	27.0
GM0	Gulf of Mexico		17	31.0
GM1	27°47′N	96°58′W	18	11.0
GM2	27°47′N	96°58′W	18	23.0
Pacific Ocean				
Sample	Location			
SN	San Nicholas Basin			29.0
SP	San Pedro Basin			32.0

TABLE 2

Collection data and vitamin B_{12} contents of short cores, Texas Bays

Sample	Latitude	Longitude	Mean depth in core (cm)	B_{12} content ng/g
Aransas Bay				
AB–0	28°02′N	97°02′W	3	7.7
AB–5	28°02′N	97°02′W	15	3.5
Sundown Bay				
SB–0	28°11′N	96°50′W	3	2.8
SB–45	28°11′N	96°50′W	47	5.7
Redfish Bay				
RB–0	27°56′N	97°06′W	3	12
RB–15	27°56′N	97°06′W	17	1.2
AC–0	27°54′N	97°07′W	3	7.0
AC–25	27°54′N	97°07′W	27	5.6
San Antonio Bay				
SAB9–0	28°26′N	96°46′W	3	9.6
SAB9–25	28°26′N	96°46′W	27	3.5
SAB9–40	28°26′N	96°46′W	42	1.2
SAB10–0	28°25′N	96°45′W	3	20
SAB10–25	28°25′N	96°45′W	27	6.3
SAB10–35	28°25′N	96°45′W	37	2.0
SAB11–0	28°23′N	96°45′W	3	16
SAB11–25	28°23′N	96°45′W	27	5.1
SAB11–35	28°23′N	96°45′W	37	3.7
SAB12–0	28°15′N	96°47′W	3	3.9
SAB12–25	28°15′N	96°47′W	27	4.6

TABLE 3

Collection data and vitamin B_{12} contents of long cores

Sample	Depth in core (cm)	B_{12} content ng/g
Redfish Bay—27°52′N, 97°05′W, 0.65 m of water		
RF–A	24–35	3.4
RF–B	92–98	0.27
RF–C	164–171	0.34
RF–D	182–189	0.31
RF–E	222–229	0.32
RF–F	404–411	0.33
Baffin Bay—27°22′N, 97°27′W, 2.5 m of water		
BBC–A	24–96	1.5
BBC–B	92–93	0.88
BBC–C	93–98	0.82
BBC–D	244–300	0.12
BBC–E	430–435	0.10
Antarctic—62°28′S, 109°07′W, 5,100 m of water		
E1	274–302	ND
E2	560–591	ND
E3	877–904	ND
E4	1193–1225	ND
E5	1502–1534	ND
E6	1814–1843	ND
Gulf of Mexico—27°47′N, 96°57′W, 20 m of water		
GC1–A	18–28	6.5
B	40–50	2.8
C	72–82	2.8
D	130–140	0.15
GC2–A	18–28	6.0
B	40–50	4.3
C	72–82	0.78
D	130–140	0.17
E	170–180	0.60
GC3–A	18–28	9.3
B	40–50	2.9
C	72–82	2.0
D	130–140	0.15
E	170–180	0.62

found in this study. Had their results been lower rather than higher, contamination might have been suspected in the analysis of our samples. There are three explanations which might account for these differences:

1. Incomplete extraction of B_{12} from our samples: This is not likely since the extraction procedures were almost the same.
2. Differences in the responses of the assay organisms to B_{12} forms: However, both organisms (17a and *Escherichia coli* mutant 113–3) have been shown to respond to a wide range of B_{12} analogues and related compounds (Van Baalen 1961; Hoff-Jorgensen 1957; Smith 1960).

3. Actual differences in the environments examined: This is possibly the best explanation since they are known to be physically different, but still the differences are remarkable. Burkholder and Burkholder (1956b) find a range of 270–870 ng of B_{12} per gram of mud. Starr (1956) gives 7–130 ng/g as a range for samples from the same area, which is somewhat closer to the range of this study—0.1–38 ng/g. Letunova (1958, 1959a, 1959b) examined muds from stagnant water reservoirs and ponds in central Russia and found B_{12} contents similar to these, *i.e.*, 3.1–163 ng/g (1958) 22–73 ng/g (1959a) and 0.5–120 ng/g (1959b).

In cores examined, B_{12} contents decreased with depth in the sediment (Tables 2 & 3). Vitamin B_{12} decreased from 1.5 ng/g at 25 cm to 0.15 ng/g at 430 cm in the Baffin Bay core and from 12 to 0.43 ng/g in the four meters of the Redfish Bay long core. It decreased from about 20 ng/g in Gulf of Mexico surface sediments to about 0.6 ng/g at 175 cm. The largest decrease in a short core was from 20 to 2.0 ng/g over 35 cm of sediment from San Antonio Bay station 10 (SAB10). This indicates that B_{12} is noticeably degraded in sediments within a geologically short time span. Thus, vitamin B_{12} does not show much promise as a biological marker in anything other than Recent sediments. Perhaps this negative evidence will be of some use. A good deal of recent organic geochemistry has been concerned with using fossil biochemicals as evidence for living organisms having been associated with various geological samples. The presence of vitamin B_{12} in such a sample (for example, a sample of lunar material) might indicate biochemical contamination.

All of the Antarctic extracts gave essentially zero B_{12} response. Since there was no growth at all, it was not possible to assign an upper limit for them. The samples themselves are probably extremely low in B_{12}, much of it having been scavenged or leached out of the detritus as it settled out through the long (5030 m) water column. These samples are geologically very old compared to the other sediments analyzed, so that B_{12} has been very much more degraded in them.

The fossil feces and bog butter (Table 4) were investigated primarily to check the preservation of B_{12} in an environment of known age and type. Since feces are known to be rich the B_{12}, it would be easily detected if it were preserved at

TABLE 4

Vitamin B_{12} and organic carbon contents of miscellaneous samples

Sample	B_{12} content ng/g	Approximate age (years)
Green River Shale	ND	50×10^6
Fossil Turkey Dung	1620	200
Fossil Human Feces	5100	200
Annelid Feces	42	fresh
Serpulid Feces	33–66	fresh
Bog Butter 1	0.14–0.28	400
Bog Butter 2	1.3	400

ND = Not detected.

all. The feces were from a dry environment, and the B_{12} is remarkably well preserved as indicated by the amount detected. The bog butter, even though buried in a moist environment probably less favorable to B_{12} preservation, still has detectable traces of the vitamin (0.2–1.3 ng/g). But it must be remembered that some of the B_{12} in these samples might be due to bacterial action which could still have taken place for a short time after deposition. Modern butter contains about 30 ng of B_{12} per gram of solids (Smith 1960).

The annelid and serpulid feces are essentially reworked detritus. They show how a sediment can become enriched in B_{12} and organic matter by passing through the guts of such organisms. In some environments, dense colonies of such animals can produce large amounts of this reworked fecal material (Frankenberg *et al.* 1967).

Some sediments were of such a nature that small amounts of interstitial water could be separated by centrifuging. The quantities recovered were not large, but their B_{12} contents were checked and found to be significant as compared to natural waters (Table 5). Sea water, for example, has been found to contain 0.0002–0.002 μg/l B_{12} depending on season and location (Cowey 1956; Daisley 1958). Vitamin B_{12} concentrations in the interstitial waters assayed ranged from essentially zero (BBC-C) to 14 μg/l with a mean content of 2 μg/l, 10^3 to 10^4 times the content of sea water.

The nutrients in interstitial water can mix with the overlying water especially in shallow water with wind agitation such as the Texas coastal bays. Thus, the sediments, with their high bacterial population, may be supplying B_{12} via interstitial water exchange to the overlying bay waters. This water exchange must be fairly rapid in some places such as San Antonio Bay, whose waters are much richer in B_{12}, than most estuaries (0.003–0.040 μg/l; L. Birke, *pers. com.*). Although high with respect to the overlying water, this interstitial B_{12} represents only 0–15% of the total B_{12} in the sediments with a mean of 2.6%. Thus, most of the B_{12} in sediment is associated with particulate matter.

The values for B_{12} contents reported in this work are necessarily minimum because:

TABLE 5

Vitamin B_{12} contents of some interstitial waters

Sample	B_{12} conc. ng/l	Percent of total B_{12} in sample
SN	0.25–0.50	1.5–3.0
GN–04	0.30–0.50	1.5–2.4
GN–010	0.49	5.6
GN–013	14	15
GM0	3.4	4.4
GR–15	0.20–0.40	0.41–0.83
GR–30	0.20–0.40	0.68–1.4
SAB10–0	1.6	4.3
SAB10–25	0.30–0.50	2.5–4.1
SAB11–25	0.20–0.40	1.4–3.9
BBC–C	<0.25	<0.85

1. 17a probably does not respond to all the B_{12} forms present in the extracts.
2. Some of the B_{12} is bound and does not diffuse as far as the standards.
3. Some B_{12} may be bound in such a way that it is not extractable from the samples.

Several extracts, the petroleum samples and Green River Shale, were found to give large zones of inhibition—45–50 mm. The inhibition was apparent not only from its overlap onto other pads but also from absence of the light green background growth usually visible on the assay plates. The shale extract also gave a colored ring which was originally thought to be a growth zone. Upon microscopic examination, however, no algal colonies were visible in these rings. The color must be due to some pigment or reaction from the extract. A few samples showed smaller zones of inhibition within the growth zones giving rise to rings similar to these but with visible algal colonies.

Errors in this work are large, but generally within the range expected for this type of investigation. Deviation among triplicate B_{12} assays of the same extract varied from 0–10% with a mean deviation of 2.3%. Deviation for triplicate extracts of the same sample ranged from 1.5–30% with a mean deviation of 12%. One of the significant sources of error in the B_{12} assay was in reading concentrations from the standard curves. This is an error inherent in any semi-log standard plot; values from the lower part of a log cycle are more accurate than those from the upper part. Variation in the volumes taken up in the filter paper discs is another source of error. Deviations between standards of the same concentration on the same assay plate can be taken as an indication of this variation, and this was slightly more than 10%. It was possible to measure growth zone diameters with vernier calipers within plus or minus 0.3 mm on successive measurements, so this did not contribute greatly to the error.

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THE PRODUCTION OF CARBON DIOXIDE BY *RANGIA CUNEATA*

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ABSTRACT

Studies of the carbon dioxide production and oxygen consumption were performed on the brackish water clam, *Rangia cuneata*. The mollusks opened and pumped almost immediately upon being placed in "fresh" water at the beginning of each determination. A burst of carbon dioxide perhaps due to discharge of accumulated bicarbonates was emitted by the clam within approximately the first 90 minutes. The production of carbon dioxide during pumping decreased in an approximate geometric fashion and was considerably greater than maximum oxygen uptake. During shell closure the amount of carbon dioxide produced was much less than during pumping, and the oxygen uptake and carbon dioxide production are roughly equivalent. The length of pumping interval shortens with lowering pH. Metabolic values ranged between 0.014 and 0.038 $mMCO_2$ per gram dry weight per hour.

INTRODUCTION

Clams of the genus *Rangia* dominate the brackish water ecosystems at the heads of estuaries in the Gulf of Mexico. According to Odum (1967) these mollusks are specialists, capable of diverting parts of their population energy budgets to salinity adaptation. They may occur in densities as high as 130 per

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square meter and are commercially important in road building in the Mississippi River Delta. The present study was begun to determine the metabolism of this important marine organism by measuring its rate of carbon dioxide production. In the course of the work it became apparent that more carbon dioxide was being produced by the clam than could be accounted for by respiratory processes alone. This paper illustrates these data and hypothesizes on their implications.

MATERIALS AND METHODS

The estuarine clam *Rangia cuneata* is found almost exclusively in bay-head facies (Ladd 1951). The specimens used in this experiment were taken from Hynes Bay (Upper San Antonio Bay) adjacent to the town of Austwell, Texas. Collections were made in water from 15 to 60 cm in depth on two occasions. The first collection was made on June 11, 1963; the salinity was 22.0 ppt. The clams were held outside in a concrete pond at a salinity 12.8 ppt. Each clam was brought into the laboratory and held for 3 days in an unaerated container (volume = 1 liter) without feeding prior to testing. Three clams from this group were designated A, B and C. The second collection was made on July 16, 1963; the salinity was 18.1 ppt. These clams were held in the laboratory in 15 liters of aerated water collected with the clams. These five clams were designated D, E, F, G and H. They were not fed during the experiments.

In determining carbon dioxide production the method of Beyers *et al.* (1963) was used. Carbon dioxide—pH titration curves were established for both types of water used in the tests (12.8 ppt and 18.1 ppt). Metabolic measurements were made using a recording pH meter.

The water used in each test was first run through a membrane filter with a pore size of 0.45μ. The water was then bubbled with air to bring it to approximate equilibrium with the atmosphere. All rates listed in the data have been corrected for the amount of water used. Clams A through F were tested in 500 ml of water, clam G in 250 ml and clam H in 80 ml. The clams were laid on their sides at the bottom of the container and no substrate was provided.

Diffusion of carbon dioxide out of the water increases in an exponential fashion with lowering of pH and varies as to the surface to volume ratio, temperature and the amount of agitation caused by the clam's siphoning action. In order to account for the amount of carbon dioxide lost by diffusion, the water was bubbled with carbon dioxide. The carbon dioxide was then allowed to diffuse out and its rate of diffusion plotted in terms of $mMCO_2$/hr at a varying range of pH values. These diffusion values were then used to find the total corrected carbon dioxide output of the clam. There was no way to account for the amount of water agitation caused by the clam's siphoning action; however, in the 500 ml samples it appeared to be negligible as the clam was well below the surface of the water.

In addition to carbon dioxide production, respiratory oxygen measurements on clams G and H were carried out in a Warburg flask with a volume of 116.2 ml. The sidearm contained 2 ml of 15% KOH. A 60 ml volume of water (18.1 ppt) was used for clam G and a 50 ml volume of the same water type used for

clam H. The water was first filtered in the same manner as in the carbon dioxide method. The flask was not shaken because the clam would not open under those conditions. An air atmosphere was used in all tests and the temperature maintained at 25.0 C.

Dry weight measurements were made by removing the body of the clam from the shell and heating at 100 C until constant weight was obtained. The shell retained the ligament.

RESULTS

Three states in the clams' activity were noted during the tests: pumping, closed, and partially open. Pumping was defined as a state in the clam's activity when its siphon was in an extended position. The shell was considered closed when no apparent gap existed between the valves. In one case (clam F) the valves remained apart approximately 3 mm. No siphon was apparent and this state is termed partially open.

A typical graph of carbon dioxide production during pumping is shown in Figure 1. The time interval of four hours was chosen because most of the clams pumped at least this long. The midpoint of each thirty minute interval was plotted except for clam H where, due to the short duration of pumping, fifteen minute intervals were used.

At the beginning of each test run the water was at its air-equilibrium pH (8.6 for clams A-C and 8.2 for clams D-H). With few exceptions, within fifteen minutes after the clam was placed in water it began pumping regardless of its state before the test. In the majority of cases upon commencement of pumping there was a very prominent burst effect in the production of carbon dioxide (Fig. 1). In all cases of pumping the rate of carbon dioxide production decreased in a roughly geometric pattern. At the beginning of each set of runs there is a marked deviation in rates between the curves, and as the runs continue the deviation becomes less and the rates more constant. At no time in any of the tests did the pH go below 7.3.

Carbon dioxide production continued even though the valves were together

TABLE 1

Carbon dioxide production in pumping and closed *Rangia*

Clam	Dry body wt (g)	Shell wt / Body wt %	Shell Length (cm)	Temperature range (C)	Average CO_2 production shell closed ($mMCO_2$/gm dry wt/hr)	Average CO_2 production shell open ($mMCO_2$/gm dry wt/hr)
A	1.446	57.5	6.68	24.2–27.2	0.0035	0.022
B	0.694	71.5	5.73	24.3–26.6	0.0058	0.038
C	1.186	80.0	6.60	22.8–25.6	0.0067	0.036
D	1.666	52.8	6.43	23.6–24.9	0.0048	0.014
E	0.829	54.8	5.60	24.0–25.2	0.0105	0.026
F	1.050	96.8	6.80	23.7–24.4	.....	....
G	0.406	42.6	4.11	25.5–26.6	Below detection	0.020
H	0.580	49.0	4.62	26.3–27.7	0.0033	0.022

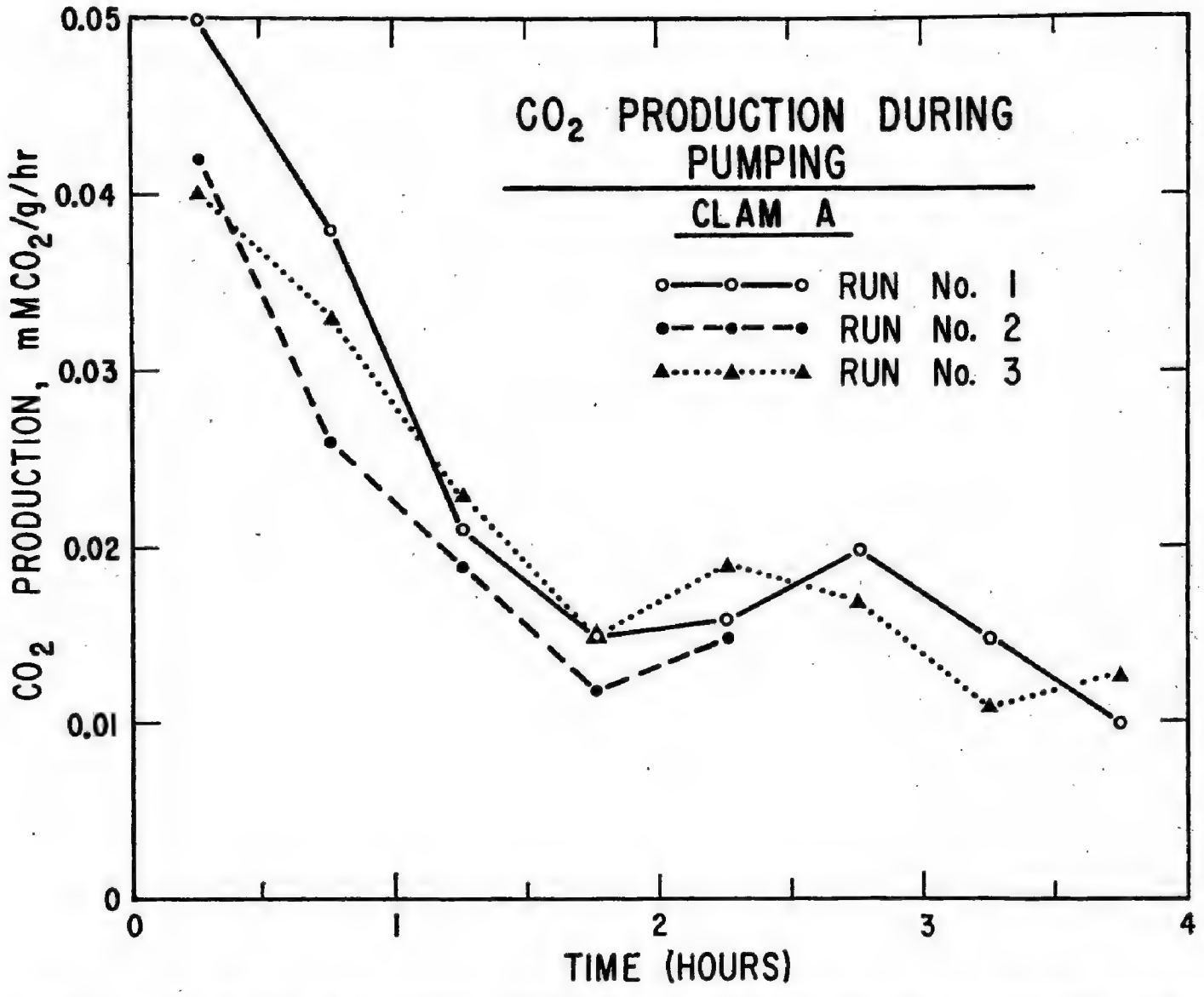

FIG. 1. The rate of carbon dioxide production in *Rangia cuneata* over a four-hour period. These clams show a burst in carbon dioxide production during the first 90 minutes after starting measurement.

(Table 1). Results of this type of measurement are most subject to error as in several cases the diffusion rate at low pH values exceeded the rate of recorded carbon dioxide production by the clam. The higher the correction factor the less accurate the data, since the diffusion rates were measured artificially by bubbling the water with carbon dioxide.

Clam F did not close its valves and this fact was utilized to determine the

TABLE 2

Oxygen consumption in *Rangia* during both pumping and closure. Temperature: 25 C. Salinity: 18.1 ppt.

Clam	Run	O_2 consumption (mMO_2/g dry wt/hr)	Volume of vessel (ml)
G	1	0.0022	60
	2	0.0019	60
H	1	0.0044	50
	2	0.0032	50

carbon dioxide production over 3–12 hour periods. Rates varied from 0.003 $mMCO_2/g/hr$ to 0.011 $mMCO_2/g/hr$. The average rate was 0.007 $mMCO_2/g/hr$.

The Warburg method of determining oxygen consumption was used for clams G and H (Table 2). Since the Warburg flask could not be shaken it was impossible to separate pumping from non-pumping rates because of the lag in oxygen diffusion. However, it is probable that the maximum rate of oxygen consumption occurred during pumping, and the minimum rate represented closure.

During pumping the average maximum rate in $mMCO_2$ per gram per hour was 0.036 for clam G and 0.061 for clam H. Comparable figures for oxygen metabolism were 0.004 and 0.005 mMO_2 respectively. This illustrates the great discrepancy between carbon dioxide production and oxygen consumption during this time. In comparison the average carbon dioxide production during closure was below detectable limits for clam G (<0.001) and 0.0033 mM carbon dioxide for clam H. The average minimum oxygen consumption was 0.009 mM and 0.0032 mM oxygen for clams G and H. There is in this limited experiment good agreement between the two methods regarding gas exchange during shell closure.

Although quantitative information on length of the pumping cycle is not included in this paper, it was apparent in the many tests performed that after the initial pumping interval had ended, the following pumping interval during the same run lasted a much shorter time. Since this occurred during the same run, the pH of the water was also much lower on the subsequent shorter pumping interval.

DISCUSSION

The fact that the clams were stimulated to pump on being placed in their test water may have been due to any combination of the following factors: higher pH, less carbon dioxide, more oxygen, lower concentration of metabolic wastes, and the effect of being moved from container to container. The very high rate of carbon dioxide production on commencement of pumping, which continued for approximately an hour and one-half, is difficult to explain by respiration alone particularly in light of the Warburg data on oxygen consumption. The maximum oxygen consumption by clam G and H was approximately 1/10 or less than that of the carbon dioxide production at the start of the test. It is reasonable to assume that, while a clam is closed, the diffusion of carbon dioxide out of its shell is restricted and that carbon dioxide tends to build up in its shell. It has been reported (Collip 1920; Dugal 1939; Revelle and Fairbridge 1957) that solution of a portion of a molluscan shell may take place under conditions of anaerobic respiration or when the animal is out of water. It is conceivable that, upon opening, a large amount of carbon dioxide is released from the shell and causes the burst effect; however, we feel there are two reasons why this is not an effect of consequence: (1) In consecutive tests in which the clams were pumping and then changed to new water, they started pumping again showing the same burst effect; (2) The length of time of the burst effect appears to be too long to be simply due to an accumulation of carbon dioxide while the shell is closed.

A possible error could be an out-put of organic acid by the clam. This would lower the pH and be interpreted as carbon dioxide production. To check on this

possibility, clams were placed in water and allowed to drop the pH. The clams were taken out, and the pH went back to normal at the same approximate rate as the rate of diffusion when the same amount of water was bubbled with carbon dioxide. Thus the drop in pH is due to carbon dioxide production rather than organic acids.

If the carbon dioxide emitted during pumping is the result of respiration, the change in rate is also rather puzzling. No change in rate was noted during pumping in the Warburg, although the time lag caused by the fact the flask could not be shaken may have accounted for this. Lack of oxygen probably was not a factor as the containers in which carbon dioxide was measured were open to the atmosphere. As further proof, one clam was placed in a vacuum jar and its atmosphere replaced by pure oxygen. The pH curve showed the same burst effect, followed by the geometric flattening of the curve.

In light of the evidence given, we propose that additional amounts of carbon dioxide are added to the water by shell building and that this accounts for the large amounts of carbon dioxide produced by the clam during pumping. The literature is very conflicting concerning the sources of calcium and carbonate ions used in shell building. According to De Waele, 1930 (as quoted by Bevelander, 1952) calcium carbonate forms as a result of the escape of carbon dioxide by physico-chemical processes. Jodrey and Wilbur (1955) suggest that at least part of the shell carbonate has its origin in the respiratory carbon dioxide. Jodrey (1953) demonstrated that dissolved calcium in sea water is taken up directly by the mantle of oysters and rapidly deposited in the shell and that the oyster mantle can deposit calcium carbonate at a relatively low rate when isolated from the remainder of the animal, indicating that some of the carbon dioxide for the precipitate is taken up directly from the sea water. Orton (1928) showed that shell growth in oysters can take place in the practical absence of food and without loss of body weight, indicating that the calcium in shells may be directly absorbed from sea water. Fox and Coe, 1943 (as quoted by Bevelander, 1952), in their studies of *Mytilus*, found that the amount of calcium obtained from food alone does not account for the amount deposited in the shell. Bevelander and Benzer (1948) demonstrated that when the calcium content of sea water is reduced, a corresponding reduction in the calcium of the growing shell results. Bevelander (1952) has shown that labeled calcium ions present in the water are ingested by the organism and are localized in several organs. In summary, we may generally assume that calcium ions in the water are taken into the tissue of the bivalve and subsequently deposited in the shell along with the possible addition of calcium ions from ingested food. The carbonate comes partly from bicarbonate ions in the water and partly from metabolic carbon dioxide, relative amounts being unknown.

If we assume the equilibrium:

$$Ca^{++} + 2HCO_3 \rightleftarrows CaCO_3 + H_2O + CO_2$$

then presumably if calcium is removed from sea water, regardless of the source of carbonate, carbon dioxide would be released. The source of the carbonate, whether derived from respiratory processes or from the water, would determine

the amount of carbon dioxide produced. As the pH became lower it might become increasingly difficult for the clam to lay down shell. According to Jodrey and Wilbur (1955) the endogenous mantle respiration of the oyster is but slightly changed by altering the pH of the sea water medium from 8.4 to 6.0. Thus the reduced rate of carbon dioxide produced during pumping may then be a function of lessened shell building rather than metabolic changes. It is doubtful that calcium becomes limiting since the pumping intervals were recorded at the beginning of each run, and 500 ml of sea water (35 ppt) contains 210 mg of calcium (Harvey 1960).

All clams except G produced a measurable amount of carbon dioxide after they closed. In two clams (G and H) the oxygen consumption approximated the carbon dioxide production. In *Rangia* around the posterior end there appears to be some mantle material projecting around the lip of the shell. In addition, around the ventral lip area of the shell are areas where small (<1 mm) gaps appear between the valves. These areas are probably the source of gas exchange during closure. Visual observations of shell movement showed that different degrees of closure exist, and this would vary the measurements. The low pH within the shell at closure probably does not allow shell deposition, and thus the carbon dioxide measured during this interval is metabolic.

Clam F did not fully close and did not appear to have a siphon. The high shell weight to body weight ratio (Table 1) indicates the clam was in poor condition at the time of the test. The average carbon dioxide production during this test was 0.007 $mMCO_2/g/hr$, which could be considered the true basal rate of this clam, whereas rates measured during closure for the other clams would be affected by the degree of closure and spacing of the valves.

The methods and equipment employed during these experiments are not sensitive enough to determine basal metabolic rates with a satisfactory degree of accuracy. In future experiments diffusion of carbon dioxide out of the water should be prevented. A kymograph or similar instrument should be used to measure shell movement during each run. The effect of shell building during pumping probably obscures metabolic rates during this interval. The ability to measure shell building by carbon dioxide production could become a useful technique especially in light of our present lack of knowledge on this subject.

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A REVISION OF THE GENUS *HOPLUNNIS* KAUP (APODES, MURAENESOCIDAE), WITH A DESCRIPTION OF A NEW SPECIES

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ABSTRACT

The genus *Hoplunnis* is redefined, primarily on the basis of osteological studies, and compared with *Muraenesox*. With the latter it shares the development of the ethmo-vomerine complex and its associated dentition and the presence of a "supraorbital" element. The species of *Hoplunnis* can be divided into two groups on the basis of the presence or absence of lateral vomerine teeth. A new species constitutes the first record of the genus from the Pacific Ocean.

INTRODUCTION

The fishes of the genus *Hoplunnis* are a well defined group of tropical marine eels. Their known range extends from the West Coast of Africa to the tropical Eastern Pacific. As construed by us, there are six valid species in the genus. The present study results from the collection of a specimen of *Hoplunnis* from Mazatlan, Mexico, which proved to be the first record of the genus from the Pacific Ocean. In addition to naming a new species of *Hoplunnis* based on the above specimen, we have attempted to bring together the existing literature on the genus

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and to study specimens of as many species as possible. Although much work remains to be done on the group, we hope that this paper will provide a basis for classification of the genus.

METHODS

Measurements were made according to the definitions given in Hubbs and Lagler (1958), except where special measurements were required for comparison with previously published data. Where these are used, they are designated by the reference points (*i.e.*, tip of snout to insertion of maxillary). Counts were made from X-rays and from the actual specimens.

The caudal portion of specimens frequently showed signs of having been broken and subsequently healed, with fin rays regenerated around the stump. Because of the gradual taper of the caudal region, a centimeter or more containing possibly twenty vertebrae and twice as many dorsal rays could easily be lost without this being obvious. For this reason the origin of the anal fin is used as the posterior reference point for vertebral and dorsal ray counts. Similarly, pre-anal length is used as the basis for the body proportions given.

Osteological data were obtained from X-rays, one cleared and stained specimen and one dissected specimen.

Dental characters were found to be of considerable value in distinguishing the species of *Hoplunnis*. The terminology involved is discussed below.

Maxillary and dentary teeth—the teeth on the maxillary and dentary bones. These teeth are referred to as biserial when arranged in two parallel rows with a distinct gap between the rows and triserial if a third row is present distally. In the latter the rows are not parallel, and the distal third row does not occupy the entire length of the jaw.

Premaxillary teeth—named by location, since the premaxillary bone is part of a fused complex including the lateral ethmoids, palatines, and vomer. They constitute a median patch of teeth at the front of the upper jaw and are separated from the maxillary and vomerine teeth by a gap.

Median vomerine teeth—a median row of large, laterally compressed, blade-like teeth in the roof of the mouth. They may be either V-shaped or lanceolate.

Lateral vomerine teeth—when present, a row of small V-shaped teeth on either side of the median vomerine teeth.

SOURCE OF MATERIAL

Specimens of *Hoplunnis tenuis*, *H. diomediana* and *H. macrura* were loaned by the U. S. National Museum (USNM). Two examples of *H. punctata* were loaned by the Congo Museum, Tervuren, Belgium, (CM). Data on the holotype of *H. punctata* were provided by the British Museum (Natural History) (BMNH), and fifty-two specimens of *Hoplunnis macrura* were loaned by The University of Texas Marine Science Institute at Port Aransas (UTMSI).

THE SPECIES OF *HOPLUNNIS*

External form and coloration are similar in all species of *Hoplunnis*, and will be treated in the generic description. The species descriptions are limited to statements of diagnosis and affinities, with Table 1 giving the counts and measurements of all the species. Collections marked with an asterisk in the distribution sections of the species descriptions are those examined by us.

Hoplunnis africanus Regan (1915) is a *nomen nudum*. This was subsequently cited by Fowler (1936) in a copy of Regan's citation. No author or description is attached to the name, and there is apparently no record of this species in the British Museum (Natural History) (Wheeler *in litt.*).

1. *Hoplunnis tenuis* Ginsburg (1951).

Hoplunnis tenuis Ginsburg (1951), p. 448, fig. 4 (Albatross Stn. 2042, 28°36′ N, 86°50′ W).
Hoplunnis schmidti (not of Kaup) Ribeiro, (1961), p. 5, fig. 3.
Diagnosis: Lateral vomerine teeth present, firmly attached to ethmo-vomerine complex not concealed by flesh of roof of mouth. Median vomerine teeth V-shaped, maxillary and dentary teeth triserial, the rows non-parallel. Visceral peritoneum black. Branchiostegals 7.

Affinities: Most closely allied to *H. diomediana*, but differing chiefly in the im-

TABLE 1

Counts and measurements of the species of *Hoplunnis*

		Pre-anal dorsal rays	Pre-anal vertebrae	Total vertebrae	Pectoral rays	Median vomerine teeth	Branchiostegals	Pre-anal length / head length	Head length / eye diameter	Head length / snout length	Head length / lower jaw length	Head length / maxillary length	Head length / distance from snout tip to maxillary articulation	Pre-anal length / depth	Pre-anal length / pectoral fin length
Hoplunnis tenuis	Max.	64	37	234	11	12	7	2.5	10.8	2.8	2.3	2.1	15.6	19.2	11.7
	Min.	60	35	191	10	7	7	2.2	9.2	2.6	2.3	2.1	11.8	13.8	10.6
	$\bar{x}$	62	36	..	10.3	9	.	2.4	10.0	2.7	..	..	13.2	17.1	11.3
Hoplunnis diomediana	Max.	70	38	228	10	10	6	2.8	11.2	2.8	2.4	2.1	15.5	18.2	16.5
	Min.	66	35	214	9	4	5	2.3	9.2	2.5	2.1	1.9	11.6	14.8	12.1
	$\bar{x}$	67.8	35.8	..	9.4	6.2	.	2.6	10.0	2.6	2.2	2.1	13.1	16.4	14.2
	Holotype (X-ray)	75	36	207	..	5	.	2.3	9.3	2.7	2.6	2.1	17.3	12.0	...
Hoplunnis punctata	Max.	69	35	..	10	8	7	2.4	9.0	2.7	2.4	2.2	16.0	13.7	9.3
	Min.	66	34	..	10	5	7	2.2	8.6	2.7	2.3	2.0	15.3	12.6	8.4
Hoplunnis macrura	Max.	71	37	240	11	10	7	2.3	10.8	2.9	2.6	2.1	23.7	13.5	14.0
	Min.	70	32	230	11	5	7	2.2	10.3	2.6	2.4	2.0	17.5	13.0	12.6
	$\bar{x}$	..	34.2	..	11	6.2	7	..	...	..	..	..	...	...	...
	Holotype	75	41	252	11	7	8	2.4	11.6	3.2	2.5	2.2	24.5	16.0	10.6
Hoplunnis pacifica															

movable lateral vomerine teeth which are not concealed by the flesh of the roof of the mouth, and in having 7 branchiostegals.

Distribution:

28°36′N; 86°50′W, Gulf of Mexico, Ginsburg (1951). (Type locality)	USNM152574.
24°14′S; 44°49′W, Sao Sebastiano Id., Brazil, Riberio (1961).	
*22°55′N; 79°27′W, 240 fms., South of Great Bahama Id.,	NSNM157942.
22°55′N; 79°16′W, 240 fms., South of Great Bahama Id.,	USNM157951.
07°26′N; 54°40′W, 95 fms., Off Surinam,	USNM185636.
09°39′N; 59°47′W, 100 fms., Off Venezuela,	USNM158895.
22°50′N; 79°08′W, 200–255 fms., South of Great Bahama Id.	USNM157946.

Material Examined: Three examples; 413 mm. submature male; 449 mm. submature female, and a 521 mm. submature female. USNM157942.

2. *Hoplunnis diomediana* Goode and Bean (1896).

Hoplunnis diomedianus Goode and Bean (1896), p. 146, pl. 43, fig. 163 (Albatross Stn. 2042, 28°36′N; 86°50′W); Jordan and Evermann (1896), p. 361; Regan (1915), p. 126; Fowler (1936), p. 274; Ginsburg (1951), p. 449.

Hoplunnis schmidti (In part, not of Kaup) Jordan and Evermann (1900), pl. 58, fig. 151 (figure only). (This figure is a copy of the illustration of the holotype of *H. diomediana* given by Goode and Bean. The mislabeling of the figure in Jordan and Evermann probably represents a lapsus).

Diagnosis: Lateral vomerine teeth present, movably attached to ethmo-vomerine complex, concealed by flesh of roof of mouth. Vomerine teeth V-shaped, maxillary and dentary teeth triserial, the rows non-parallel, visceral peritoneum black, branchiostegals 5 or 6.

Affinities: Closest to *H. tenuis*, from which it differs primarily in the movable and concealed lateral vomerine teeth and in having 5 or 6 branchiostegals. This species and *H. tenuis* form a distinct unit within the genus *Hoplunnis*. They differ from the remaining species primarily in the presence of palatine teeth, the V-shaped median vomerine teeth, the triserial maxillary and dentary teeth, and the black visceral peritoneum.

Distribution:

28°36′N; 86°50′W, Gulf of Mexico, Goode and Bean (1895) (Type locality).	USNM44240.
*28°09′N; 84°54′W, 80 fms., West of Tampa, Fla.	USNM163519.
29°09′N; 85°59′W, 110 fms., Off Cape San Blas, Fla.	USNM158152.
29°10′N; 85°48′W, 55–71 fms., Off Alabama Coast	USNM185635.
27°39′N; 79°54′W, 100 fms., Off East Coast of Fla.	USNM195906.

Material Examined: Ten specimens; five males with mature testes, 280 mm., 290 mm., 297 mm., 331 mm., 360 mm.; five females with mature ovaries; 381 mm., 383 mm., 414 mm., 428 mm., 436 mm., USNM163519.

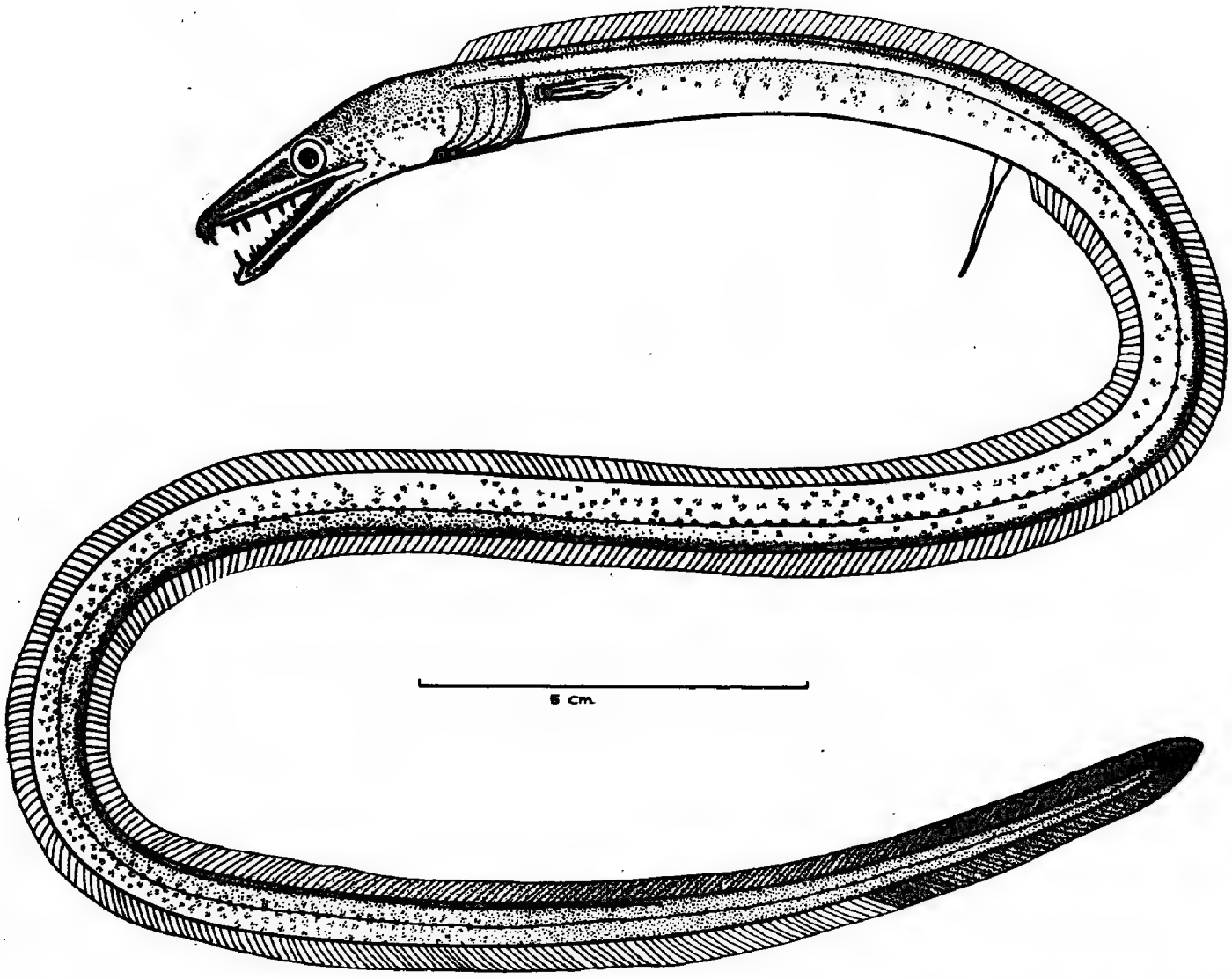

FIG. 1. *Hoplunnis pacifica* sp. nov. holotype, standard length 470 mm., mature female. It is not known whether the fleshy appendage at the vent is accidentally torn and extruded intestine or a functional organ, perhaps for egg deposition.

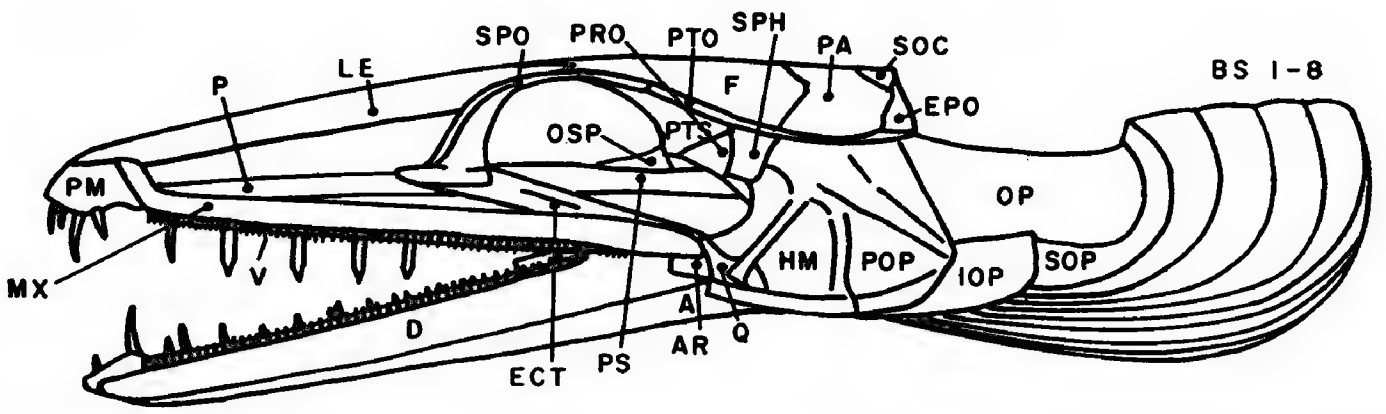

FIG. 2. *Hoplunnis pacifica*, diagram of right side of skull (reversed). PM, premaxillary plate; LE, ethmo-vomerine complex (fused with premaxillary plate); SPO, "supraorbital," F, frontal; PA, parietal; SOC, supraoccipital; PTS, pterosphenoid; PRO, prootic; PTO, pterotic; SPH, sphenotic; EPO, epiotic; MX, maxillary; D, dentary; ECT, ectopterygoid; Q, quadrate; HM, hyomandibular; POP, preopercular; OP, opercular; SOP, subopercular; IOP, interopercular; BS, branchiostegals.

3. *Hoplunnis pacifica* sp. nov. (Figs. 1 & 2).

Diagnosis: Lateral vomerine teeth absent, median vomerine teeth lanceolate. Maxillary and dentary teeth biserial, the rows parallel. Visceral peritoneum colorless, branchiostegals 8. Distance from tip of snout to articulation of maxillary with ethmo-vomerine complex 24.5 in head, eye diameter 11.6 in head. Preanal vertebrae 41.

Affinities: *H. pacifica* seems to be most closely allied with *H. macrura*, with which it shares most features except the high number of vertebrae and branchiostegal rays.

Holotype: A 470 mm. female with mature ovaries containing free eggs about 1 mm. in diameter. BC61–169, Mazatlan, Mexico, caught in webbing of shrimp trawl. Exact locality of capture unknown. Counts and measurements are given in Table 1, and the specimen is illustrated in Figures 1 and 2. This is the only known example of the species. This species is named *pacifica* since it is the only known *Hoplunnis* species from the Pacific Ocean.

4. *Hoplunnis macrura* Ginsburg (1951).

Hoplunnis macrura Ginsburg (1951), p. 450, fig. 5.

Diagnosis: Lateral vomerine teeth absent, median vomerine teeth lanceolate. Maxillary and dentary teeth biserial, the rows parallel. Visceral peritoneum colorless, branchiostegals 7. Distance from tip of snout to anterior-most maxillary tooth 17.5–23.7 in head, eye diameter 10.3–10.8 in head. Preanal vertebrae 32–37.

Affinities: *H. macrura* seems to be intermediate between *H. punctata* and *H. pacifica*. It agrees with the former in vertebral count and branchiostegal number, and with the latter in the distance of the anterior-most maxillary teeth from the tip of the snout, and the eye diameter.

Distribution:

29°14′N.; 88°35′W, Off Mississippi Delta (Type locality)	USNM152565
29°11′N.; 88°50′W, Off Mississippi Delta (Paratype)	USNM152566
*06°40′N.; 54°25′W, Off Surinam	USNM185661
28°20′N.; 90°? ′W, 19 fms., South of Louisiana Coast	USNM158891
27°27′N.; 78°58′W, 80 fms., North of West Palm Beach, Florida	USNM158320
27°33′N.; 96°24′W, 97 fms., South of Corpus Christi, Texas	USNM157945
29°20′N.; 88°07′W, 120 fms., Off Mississippi Coast	USNM186203
06°44′N.; 55°33′W, 25 fms., Off Surinam	USNM158896
29°42′N.; 88°29′W, 20 fms., Off Mobile, Alabama	USNM158255
"Directly South of Houma, Louisiana"	USNM162575
*"Off Punta Frontera, Mexico, 13–20 fms."	UTMSI
*"60–70 miles south of Port Aransas, Texas, 18–23 fms."	UTMSI
*"40–70 miles south of Port Aransas, Texas, 12–18 fms."	UTMSI

Material Examined: Four collections, the first with two examples, 484 mm. ripe female and 441 mm. ripe female, USNM185661. The remaining three collections containing together 52 specimens, 159–388 mm. The 159 mm individual

apparently was a newly metamorphosed juvenile; the remainder of the collection is made up of submature and mature specimens of both sexes.

5. *Hoplunnis punctata* Regan (1915).

Hoplunnis punctatus Regan (1915), p. 126; Fowler (1936), p. 274.
Hoplunnis diomedianus (not of Goode and Bean) Poll (1953), p. 135, fig. 53.

Diagnosis: Lateral vomerine teeth absent, median vomerine teeth lanceolate. Maxillary and dentary teeth biserial, the rows parallel. Visceral peritoneum colorless, branchiostegals 7. Distance from tip of snout to anterior-most maxillary tooth 15.3–16.0 in head, eye diameter 8.6–9.0 in head. (Dr. Max Poll kindly lent us two of the series of ten *Hoplunnis* he reported (op. cit.) as *diomediana*. They agree with an X-ray and data sent us by Dr. A. C. Wheeler of the British Museum (Natural History) on the holotype of *H. punctata*.)

Affinities: *H. punctata* belongs with *H. macrura*, *H. schmidti*, and *H. pacifica* in the second group of species within the genus *Hoplunnis*. Of these it would seem to be closest to *H. macrura*, since the two species agree in branchiostegal count (7) and in having 32–36 preanal vertebrae. In two features, the large eye size and the more posterior location of the articulation of the maxillary to the ethmovomerine complex, *H. punctata* falls within the range of *H. tenuis* and *H. diomediana*.

Distribution:
*Lagos, West Africa, Regan (1915) (Type locality).
*6°10′S.; 11°35′E, West by South of Moita Secc., Congo, Poll (1953) C.M.

Material Examined: X-ray and data on Holotoype, a 370 mm. example of unknown sex (Wheeler *in litt.*), and two examples from the Congo Museum series; 393 mm. and 368 mm. males with mature testes.

6. *Hoplunnis schmidti* Kaup (1859).

Hoplunnis schmidti Kaup (1859), p. 19, pl. 2, fig. 4; Gunther (1870), p. 49; Jordan and Evermann (1896), p. 361; Regan (1915), p. 126; Meek and Hildebrand (1923), p. 143; Fowler (1936), p. 274; Ginsburg (1951), p. 447.

The holotype and only known specimen of *H. schmidti* was probably destroyed during World War II (Ladiges *in litt.*). The specimen reported by Ribeiro (1961) agrees with *H. tenuis*, but not with the original description of *H. schmidti*. All data given below are taken from the original description of *H. schmidti*.

Diagnosis: Lateral vomerine teeth absent, median vomerine teeth lanceolate. Ginsburg (1951) says *H. schmidti* has lateral vomerine teeth, but Kaup's description translates as follows: "On the vomer are ten long, transparent teeth, milk-white at their bases, lanceolate at the point, thicker and longer than all the rest. These teeth are very deep-rooted and are not accompanied laterally by lengthwise rows of small teeth, as is shown in *Muraenesox*."

Affinities: Kaup's description gives sufficient data to place *H. schmidti* with *H. punctata*, *H. macrura* and *H. pacifica*, but is not adequate to separate it from any

of these. *H. schmidti* was described from a specimen from Puerto Cabello, Venezuela, which places it within the range of *H. macrura*. We have examined 52 specimens of *H. macrura* from the Coast of Mexico, near the mouth of the Rio Grande, and two from Surinam. There is no apparent reason for separating these specimens into two species. This strongly suggests that *H. macrura* should be placed in the synonymy of *H. schmidti*. Without any knowledge of the holotype of *H. schmidti* beyond Kaup's description and without any specimens of *Hoplunnis* from the type locality of *H. schmidti*, we hesitate to take this step. Therefore, we provisionally maintain *H. macrura* as a distinct species to be placed near *H. schmidti*.

Distribution: Puerto Cabello, Venezuela, Kaup (1859) (Type locality).

KEY TO THE KNOWN SPECIES OF *HOPLUNNIS*

A. Lateral vomerine teeth present on either side of median vomerine teeth, median vomerine teeth V-shaped. Maxillary and dentary teeth in three nonparallel rows, the outer-most row shorter than the two inner rows (Fig. 3). Visceral peritoneum black. Distance from tip of snout to anterior-most maxillary tooth 11.6–16.8 in head.

I Lateral vomerine teeth projecting above flesh of roof of mouth and firmly attached to ethmo-vomerine complex. Branchiostegals 7.
Hoplunnis tenuis, Ginsburg (1951).

II Lateral vomerine teeth concealed by flesh of roof of mouth and movable, not firmly attached to ethmo-vomerine complex. Branchiostegals 5–6.
Hoplunnis diomediana, Goode and Bean (1896).

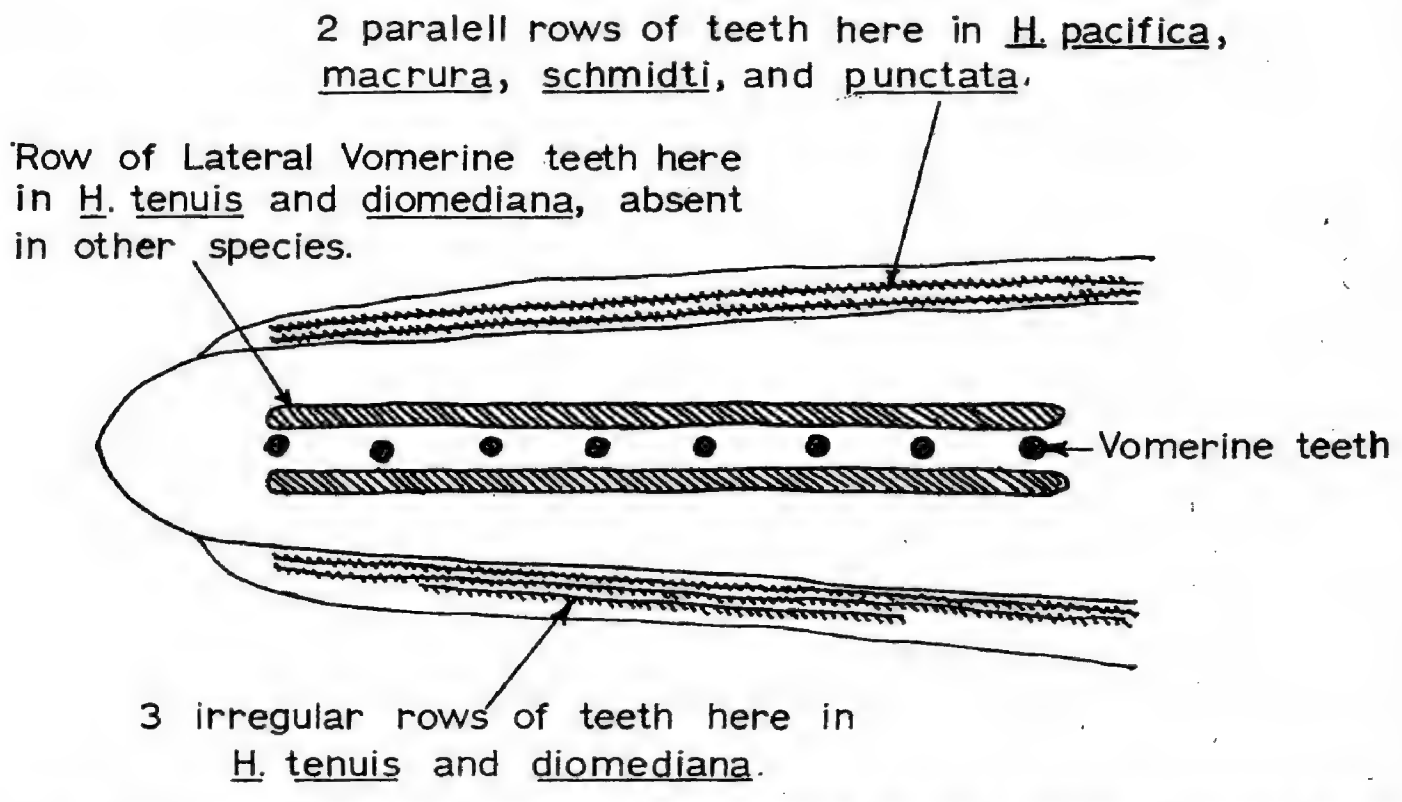
2 paralell rows of teeth here in H. pacifica, macrura, schmidti, and punctata.
Row of Lateral Vomerine teeth here in H. tenuis and diomediana, absent in other species.
Vomerine teeth
3 irregular rows of teeth here in H. tenuis and diomediana.

FIG. 3. Sketch of the upper jaw of *Hoplunnis* showing the tooth patterns. (Patterns similar on lower jaw.)

B. Lateral vomerine teeth absent, median vomerine teeth lanceolate. Maxillary and dentary teeth in two parallel rows with a distinct gap separating the rows. Visceral peritoneum transparent. Distance from tip of snout to anterior-most maxillary tooth 15.3 to 24.5 in head.

I Distance from tip of snout to anterior-most maxillary tooth 15.3–16.0 in head, eye diameter 8.6–9.0 in head.

Hoplunnis punctata, Regan (1915).

II Distance from tip of snout to anterior-most maxillary tooth 17.5–24.5 in head, eye, diameter 9.2–11.6 in head.

a. 32–37 vertebrae before insertion of anal fin. Branchiostegals 7. Eye diameter 10.3–10.8 in head.

Hoplunnis macrura, Ginsburg (1951).

Hoplunnis schmidti, Kaup (1859).

b. 41 vertebrae before insertion of anal fin. Branchiostegals 8. Eye diameter 11.6 in head.

Hoplunnis pacifica sp. nov.

GENUS *HOPLUNNIS*, Kaup, 1859

Hoplunnis, Kaup (1859), p. 19, pl. 2, fig. 4. (Type species *H. schmidti* by original designation, Kaup 1859).

External Features

Body long, slender, tapering behind vent to a laterally compressed, pointed tail.

Head long, 2.2–2.9 in preanal length, the jaws elongate, extending behind eye, 2.1–2.6 in head. Mouth terminal, upper jaw extending slightly in front of lower jaw. Eye rather large (for an eel), its diameter contained 9–12 times in head length. Gill opening a vertical slit, restricted to lower ½ to ⅔ of lateral surface of body. Anterior nostrils tubular, located near tip of snout. Posterior nostrils horizontal slits, just anterior to orbits on sides of head.

Dorsal and anal fins delicate, with numerous articulated, but unbranched, rays. In *H. pacifica*, the dorsal fin contained approximately 394–397 rays; the previously mentioned difficulty with respect to loss of the posterior of the fish makes this uncertain, therefore total rays are not used. Dorsal originating in advance of gill opening, continuous with caudal. Anal originating immediately posterior to vent and confluent with caudal. Pectoral fins well developed, with 9–12 branched rays, their length contained 8–15 times in the preanal length.

Lateral line double, a dorsal component beginning just behind and beneath dorsal origin and extending to posterior ⅓ of postanal portion of body. The ventral component begins at level of posterior margin of opercular and extends to tip of tail.

Color in life, bright silver laterally, in at least *H. diomediana*, *H. macrura* and *H. schmidti* (Kaup 1959). Some preserved specimens seen by us retain an opalescent sheen on the lateral body surfaces. Color in preservative generally yellowish or pinkish brown on lateral surfaces, darker above and flesh-colored ventrally. Scattered melanophores present on sides of body as shown in Figure 1.

Dentition

Maxillary and dentary teeth, except those in symphyseal region of dentary, triangular and laterally compressed. In *Hoplunnis punctata*, *H. macrura*, *H. schmidti* and *H. pacifica* they are in two parallel rows, with a distinct gap between the rows. In *H. tenuis* and *H. diomediana*, they are in three non-parallel rows with an indistinct gap separating the rows. The outer row shorter than the two inner rows. In all species, the teeth of the inner row are almost twice the size of those in the outer rows and more widely spaced.

Teeth of the symphyseal region of the dentary are elongate, conical and recurved; the two lateral teeth apparently fit on outside of upper jaw when mouth closed. [Kaup (1859) says this is not so in living specimens.]

Premaxillary teeth similar in form to those of symphyseal region of dentary and separated from maxillary, palatine and vomerine teeth by a large gap.

Median vomerine teeth (except the first anterior tooth) laterally compressed, dagger-like, either V-shaped, as in *H. tenuis* and *H. diomediana*, or lanceolate as in *H. punctata*, *H. macrura*, *H. pacifica*, and *H. schmidti*. Anterior-most tooth variable, but usually conical, recurved and elongate, as are the premaxillary teeth.

Lateral vomerine teeth present only in *H. tenuis* and *H. diomediana*. They are laterally compressed and V-shaped, forming a row on either side of the median vomerine teeth. In *H. diomediana*, they are loosely attached to the bone of the roof of the mouth and mostly covered by epidermis, whereas in *H. tenuis* they are strongly attached to the bone of the roof of the mouth and not covered by epidermis.

Osteology

The osteology of six species in four related genera was studied. These were: (1) *Saurenchelys gilberti* (Nettastomidae), (2) *Ariosoma prorigira* (Congridae), (3) *Muraenesox coniceps* and *M. arabicus*, and (4) *Hoplunnis macrura* and *H. pacifica* (Muraenesocidae).

The skull of *Hoplunnis pacifica* is illustrated in Figure 2. Labeling of the drawing and osteological terms follow Harrington (1955).

The antorbital region is much elongated in comparison to *Ariosoma* and *Muraenesox*. The lateral ethmoids, premaxillary, vomer, and possibly auto-palatines are fused into a single complex resembling an I-beam in cross-section. The same sort of fusion is present in *Muraenesox*, but in *Ariosoma* and other congrids there is an open area between the ventral surface of the ethmoids and the dorsal surface of the vomer (Asano 1962). Unlike *Muraenesox* or *Ariosoma*, there were no nasal bones in *Hoplunnis*.

The orbit is enclosed dorsally and anteriorly by an element we term the "supraorbital." At its posterior dorsal extremity it is sutured to the pterotic, and it curves around the dorsal and anterior portions of the orbit, ending in an anvil-shaped enlargement at its anterior-ventral extremity. A similar structure is present in *Muraenesox*, but is restricted to the anterior margin of the orbit and does

not meet the pterotic dorso-posteriorly. No similar structure is present in any congrids known to the authors, nor is it present in *Saurenchelys*.

Morphological evidence is not sufficient to determine the origin or homologies of this structure, and embryological data are lacking. The lateral ethmoid processes seen in the congrid subfamily Anagoinae (Asano 1962) are placed far in advance of the orbit and are fused with, rather than sutured to, the lateral ethmoids. Direct homology with the supraorbital components of the circumorbital bones also seems unlikely, since these are largely lost (by fusion) in apodal fishes. Gregory (1933) shows a suborbital ring in *Gymnothorax funebris* in which the supraorbital components are represented only by the fused parethmoid and prefrontal. This complex, however, does not extend far enough posteriorly to meet the pterotics, and the series would have to be complete through the postfrontal to do so. To our knowledge, the supraorbital and postfrontal bones, as such, are not present in apodal fishes. While we can offer no satisfactory explanation of the homology of this bone, its presence in both *Hoplunnis* and *Muraenesox* would seem to indicate the close relationship of these forms.

Posterior to the orbital region, the skull does not show the great elongation seen in the antorbital portion. The frontals are ankylosed for their entire length, as is true of all eel groups near the congrid stem (Berg 1947). In both *Hoplunnis* and *Muraenesox*, the pterotics extend onto the dorsal surface of the skull to about the same extent as in *Saurenchelys*.

The suspensorium is strong and directed somewhat forward of the vertical. The hyomandibular is the largest single element in the suspensorium. The quadrate is restricted to a small area just above the articulation of the lower jaw. The symplectic (not visible in the illustration) is a splint-like bone on the anterior face of the large vertical strut of the hyomandibular. The metapterygoid is not apparent in *Hoplunnis*, possibly being lost by fusion with the hyomandibular. The remainder of the pterygoid series is represented only by the ectopterygoid, which is a narrow, flattened bone, running from the anterior margin of the hyomandibular—metapterygoid complex, just above the jaw articulation to the ventral-lateral angle of the ethmo-vomerine complex, near its posterior extremity.

The jaws have already been described to some extent in connection with the dentition. The maxillary is a long, slender bone, articulating with the ethmoid anteriorly at a point much further forward than in *Muraenesox*. No supplemental bone is present. The dentary makes up most of the lower jaw. The only accessory bone present is the angular, on the medial surface of the dentary. It is a long, narrow element, enlarged posteriorly, at the location of the facet for articulation with the quadrate.

The opercular series is complete and shows little reduction when compared with more specialized eel groups such as the ophichthids. The preopercular is a heavy bone, sutured anteriorly to the hyomandibular. The opercular is smaller, and consists of a nearly vertical rod-like process extending ventrally from its articulation with the epiotic, to terminate as a small, flattened extension lying just above the interopercular and subopercular.

There are five to eight branchiostegals in *Hoplunnis*, the number being of

value in distinguishing the species. They are elongate, exceedingly fine rods as is typical of eels. In *Muraenesox*, the branchiostegals are more numerous (18 in *M. arabicus*).

Hoplunnis shares the following features with *Muraenesox*: (1) antorbital region and jaws elongated; (2) antorbital complex of bones solidly fused into an I-beam-like structure; (3) vomerine teeth bladelike, greatly developed; (4) presence of a "supraorbital" element; and (5) caudal vertebrae without lateral processes on the haemal arches.

They differ from *Muraenesox* in the following respects: (1) the maxillary articulated with the ethmoid much further forward in *Hoplunnis*, (2) the branchiostegals are much less numerous in *Hoplunnis*, and (3) *Hoplunnis* lacks nasal bones. *Hoplunnis* and *Muraenesox* agree with *Saurenchelys* only in the tendency for the pterotic to extend onto the skull roof and in the lack of transverse processes on the haemal arches. There is no "supraorbital" in *Saurenchelys*, and the antorbital complex, although showing fusion, is much reduced and lacks the I-beam structure.

As pointed out earlier, *Hoplunnis* and the congrid group are widely divergent in skull structure. The chief points of difference are: (1) the open area in the antorbital complex between the lateral ethmoid component dorsally and the vomero-palatine component ventrally in the congrids, (2) the lack of a supraorbital bone, (3) the pterotic does not extend onto the roof of the skull in the congrids, and (4) transverse processes are present on the haemal arches of the caudal vertebrae in congrids.

On the basis of the above data, the authors suggest that the Nettastomidae, *Hoplunnis*, *Muraenesox* and a number of other genera not examined by us, constitute one line of evolution derived from the congrid stem. Until genera such as *Xenomystax*, *Neoconger*, *Gordiichthys* and *Leptoconger* are examined, the exact pattern of this evolutionary trend cannot be determined. From our data, it would seem that *Muraenesox* is the most congrid-like of these forms, and the Nettastomidae, as a group, are the least. *Hoplunnis* certainly belongs with *Muraenesox* in the family Muraenesocidae, which we provisionally define as follows:

Eels with the frontals ankylosed for their entire length. Antorbital region of skull elongate, its bones fused into an I-beam-like structure with no opening between the ethmoid and vomero-palatine areas. Maxillaries articulating with ethmoids only distinctly posterior to premaxillary region of antorbital complex. "Supraorbital" bone present, pterotic extending onto skull roof. Caudal vertebrae without transverse processes on the haemal arches. Vomerine teeth specialized as enlarged blade-like teeth, and jaws elongate.

EVOLUTION IN *HOPLUNNIS*

The genus *Hoplunnis* is divisible into two groups of species. *H. tenuis* and *H. diomediana* belong in one group, and share the following features: lateral vomerine teeth present; median vomerine teeth V-shaped; maxillary and dentary teeth triserial, in non-parallel rows; and articulation of maxillary with ethmo-

vomerine complex remote from snout. These characters are all closer to the condition seen in *Muraenesox* than they are in the remaining species of *Hoplunnis*. Of the two species, *H. tenuis* seems to be more *Muraenesox*-like in that the lateral vomerine teeth are better developed, and the branchiostegals are greater in number than in *diomediana*. We suggest, therefore, that the *H. tenuis—H. diomediana* group is the more primitive of the two, and that *H. tenuis* is more primitive than *H. diomediana*.

The second group of species contains *H. punctata, H. schmidti, H. macrura* and *H. pacifica*. This group shows a number of dental specializations. The lateral vomerine teeth are lost, the median vomerine teeth are lanceolate, rather than V-shaped, and the maxillary and dentary teeth show a more fixed arrangement into two parallel rows. The primary differences between *Hoplunnis* and *Muraenesox* are dental specializations, and this group extends those specializations further along the same lines which were started in the *tenuis-diomediana* group. *H. punctata* shows all of the dental specializations seen in the second group, but retains the large eye size and posterior maxillary articulation of the first group. This species may be considered the basal member of the second group. *H. macrura* and *H. pacifica* have attained about the same level of specialization with respect to all characters so far considered. However, the number of vertebrae is greater in *H. pacifica* than in all other species of *Hoplunnis*, and there is one more branchiostegal. We suggest that *H. pacifica* is a derivative of *H. macrura* which became isolated at the time the continents of North and South America became joined.

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STUDIES ON THE REFLECTING LAYERS OF FISHES

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ABSTRACT

Samples of tissues that were brightly reflecting were collected from many fishes occupying a great variety of environments, and the tissues were examined for guanine. An enzyme method of assay was employed, making use of xanthine oxidase and guanase. Fishes collected were elasmobranchs, chimaerids and teleosts. Environments ranged from shallow inshore to bathybenthic. Shiny tissues were skin, iris, peritoneum and chorioid. Levels of guanine in the skin ranged from about 25 μg per cm^2 in fishes having a slight sheen such as *Stomias*, to 700 μg per cm^2 in very silvery fishes such as the cutlass fish. Chorioidal tapeta lucida contained high levels of guanine, over 900 μg per cm^2 in the stingaree. Preparations of clean crystals were obtained from the skin of the cutlass fish and the chorioid of the stingaree, and shown to be guanine. Chromatograms revealed, in addition to guanine, hypoxanthine and uric acid in preparations from the cutlass fish and several fluorescent substances one of which, from the chorioid of the stingaree, was identified as isoxanthopterin. Shiny tissues of squid, on the other hand, lack guanine. Some optical properties of the skin of the cutlass fish are described, and some comments are ventured about the role of guanine in vision and camouflage.

INTRODUCTION

Many fishes are silvery because of shiny layers in the skin. Shiny or lustrous surfaces occur in other places as well, in internal cavities, especially the abdomen; in the iris; surrounding the eyeball and red muscle masses; and in ocular tapeta lucida (Cunningham and MacMunn 1894; Denton and Nicol 1966). The shiny exteriors of silvery fishes such as herring make for camouflage, concealing the

fish and rendering it difficult to see under conditions of subaquatic illumination (Franz 1907; Denton and Nicol 1965). Similarly, an internal reflecting layer, such as the stratum argenteum that envelopes red muscle masses, serves to hide an internal structure from external view (Cunningham and MacMunn 1894). Ocular tapeta lucida play a role in vision in nocturnal animals and in those that live under conditions of poor illumination (Walls 1942).

The shiny reflecting layers of fishes contain guanine, and it is generally held that the shine or lustre is caused by platelets or crystals of guanine within the tissues (Millott 1923; Sumner 1944; Neckel 1954; Nicol 1963; Best and Nicol 1967).

The present study originated from a consideration of the influence of habitat on the degree of development of reflecting layers. It was conjectured that fish from different environments, subject to different degrees of illumination, would possess different amounts of reflecting material. Therefore, samples of tissues were collected from diverse fishes to determine amounts of guanine present. The results of these measurements are presented, correlations are made, and conclusions are drawn relating to environmental illumination and reflectivity. The character of the reflecting material is considered more thoroughly, using tissues from a few favorable and accessible species. Also examined were tissues from squid which possess silvery reflecting layers as prominent as those of shiny fish.

This field is one that deserves more attention than it has hitherto received, because of the functional importance of the tissues to the survival of the animal, and because of the many interesting and complex biophysical and biochemical problems posed by the highly organized reflecting cells.

MATERIALS AND METHODS

Fishes were obtained from many places and sources: in inshore waters, by usual collecting methods, such as otter trawl and beach seine; in oceanic waters by long-lining, Isaacs-Kidd trawl or one-meter net and dip nets; and from a fish hatchery (which supplied coho salmon smolts).

Tissues of oceanic species were secured during two oceanic cruises, *i.e.*, one by R. V. Sarsia of the Marine Biological Association U. K. in June 1966 to the Bay of Biscay, and one by R. V. Yaquina of the Department of Oceanography of Oregon State University in February 1967 off the coast of Oregon.

For assays, pieces of known surface-area of skin or other tissue were taken, usually by punch-biopsy, using a cork-borer. Tissues were frozen or, in one series, dried at 105 C, and stored frozen or dried, respectively.

The enzymes were purchased from Sigma Chemical Co., St. Louis, Mo., and from Worthington Biochemicals, Inc., Freehold, New Jersey. All other chemicals were of reagent grade. We are indebted to Dr. Hugh S. Forrest for samples of xanthopterin sulfate and erythropterin.

Guanine-assay. Guanine-content was determined by enzyme assay (Shuster 1955). The tissue was steeped in 0.1 N NaOH (usually 2 ml) for 24 to 48 hours. The extract was centrifuged, if necessary, and the volume or weight of fluid recorded. An aliquot (usually 0.1 ml) was drawn and added to Tris (Tris(hydro-

xymethyl)aminomethane) buffer pH 8.1, final volume 3 ml. The spectrum of the crude extract in Tris was determined in a recording spectrophotometer (Bausch and Lomb 505). Small amounts of xanthine oxidase (0.010 ml) and guanase (0.015 ml) were then added to the buffered sample and the increase of absorbance, if any, at 290 nm was determined (290 nm is the wavelength of maximal absorbance of uric acid). In some instances a small amount of uricase (0.02 ml) was then added, and decrease of absorbance at 290 nm was followed, as the uric acid was converted to allantoin.

A standard solution of guanine containing 10 μg per ml was prepared. After addition of xanthine oxidase and guanase the increase of absorbance at 290 nm was 0.55. This figure was used for estimating guanine content of tissue extracts.

Rational. Guanine in Tris buffer (pH 8.1) has absorption peaks at 243 and 272 nm; xanthine, at 238 and 273 nm; hypoxanthine, at 247 nm; and uric acid, at 233 and 290 nm.

The addition of xanthine oxidase to the sample converts any xanthine or hypoxanthine present to uric acid. Guanase subsequently added converts guanine to xanthine, and xanthine oxidase converts the latter to uric acid. The increase of density at 290 nm is a measure of the amount of guanine present in the sample (Fig. 1).

Uricase converts uric acid to allantoin, and the density at 290 nm decreases.

Example. A circle of skin from a ribbon fish had an area of 3.45 cm^2. It was steeped in 3 ml of 0.1 N NaOH for 2 days. The extract was centrifuged, an aliquot of 0.03 ml was drawn and added to Tris buffer to a final volume of 3 ml. An absorbance curve from 200 to 350 nm was determined. Xanthine oxidase, 0.01 ml, was added, and the density at 290 nm was immediately noted. There was no further increase of density. Guanase, 0.015 ml was added, and again the initial density was recorded at 290 nm. Changes of density at 290 nm were noted at frequent intervals until no further change occurred (20 min), and a final absorbance curve was secured. Uricase, 0.01 ml, was added and decrease of density at 290 nm was followed. A final absorption curve was obtained.

Increase of absorbance at 290 nm consequent upon addition of xanthine oxidase and guanase was 0.45.

A. 1 ml of the solution measured therefore contained
$$\frac{0.45}{0.55} \times 10 = 8.2 \ \mu\text{g guanine}$$
B. 3 ml of the solution contained 24.6 μg guanine
C. 3 ml of extract contained $\dfrac{24.6 \times 3}{0.03} = 2460 \ \mu$g guanine.
D. Unit area of skin, 1 cm^2, contained
$$\frac{2.46}{3.45} = 0.72 \text{ mg guanine}$$

OBSERVATIONS AND RESULTS

The sections that follow present the results of the assays for guanine, descriptions of two trials to obtain clear crystals and to characterize them, investigations

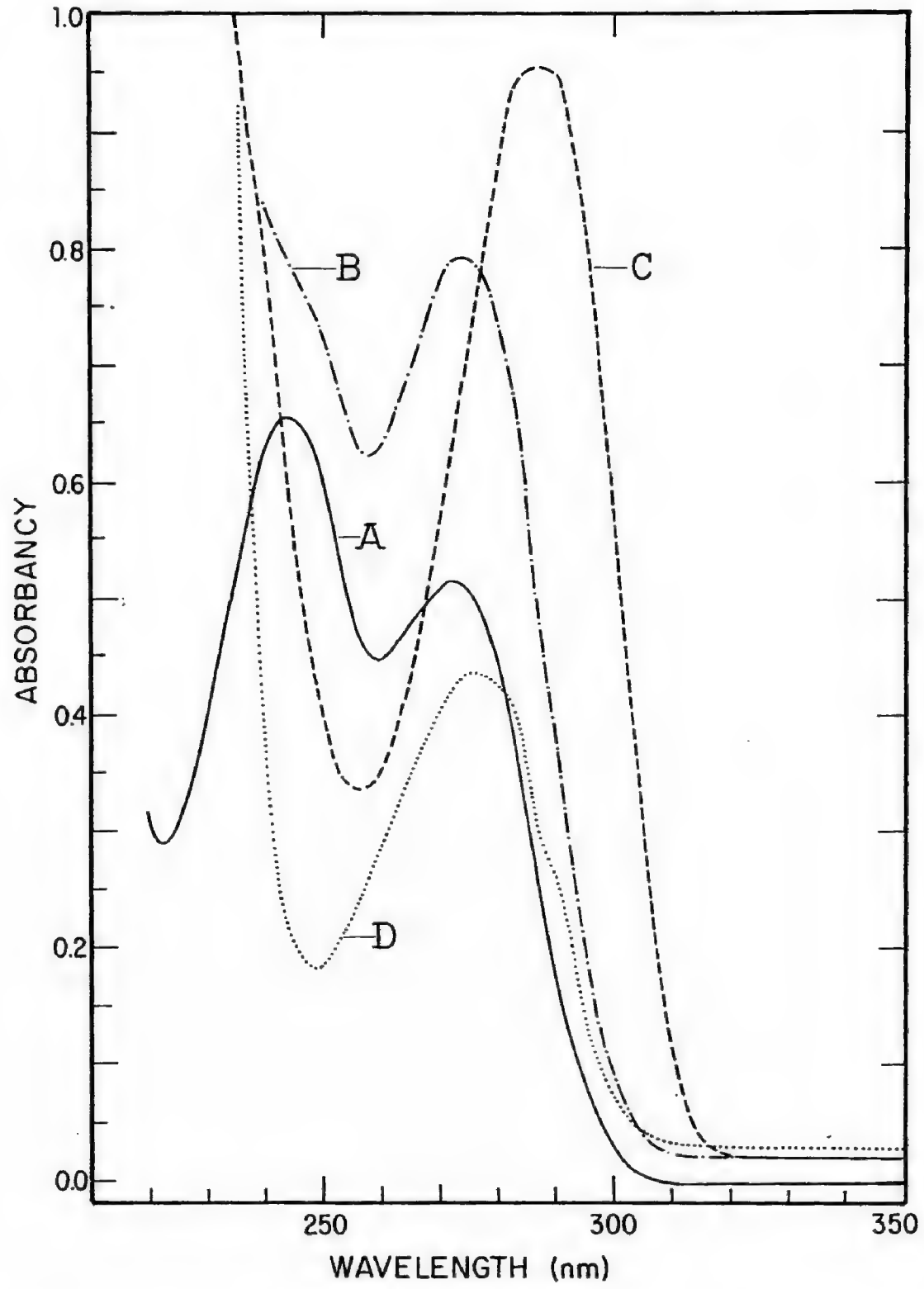

FIG. 1. Absorption spectrum of guanine and its conversion to uric acid. A, guanine, 10 μg/ml in Tris buffer (pH 8). B, absorbance immediately after addition of xanthine oxidase and guanase. C, final curve, conversion to uric acid. D, an absorbance curve for xanthine oxidase and guanase in approximately the same concentration as that used in the assay. No final curve after addition of uricase.

of tissues and of crystals with paper chromatography, and observations on the silvery surfaces of some fishes.

Guanine Content

Four kinds of tissues were examined and assayed for guanine; they were the skin, iris, peritoneum and tapetum lucidum. The fishes were benthic and nektonic species of inshore and coastal waters; pelagic oceanic, either of the upper

layers (epipelagic) or of middle layers (mesopelagic); bathypelagic; batl benthopelagic (*i.e.*, deep-water species living near the bottom); and bathybentl The results are presented in Table 1. Selected absorbance curves of extracts shown in Figures 2 to 8.

TABLE 1

Amounts of guanine in fish tissues expressed as weight of guanine in a unit surface-area

Fish	Preparation	Guanine mg per cm²
Section 1. Skin and Peritoneum		
Ribbon or cutlass fish	Silvery skin of lateral flank	0.661, 0.718
Trichiurus lepturus (a)	Lower flank near tail	0.351
"	Operculum (outer plus inner surfaces)	0.387
"	Dorsal fin	Negative
Croaker *Micropogon undulatus* (a)	Skin of silvery flank	0.150, 0.158, 0.175, 0.238, 0.268
Cigar or lizard fish	Dorsum, brown skin behind dorsal fin	0.022
Synodus foetens (a)	Dorsum, brown skin below dorsal fin	0.105
"	Silvery skin of lateral flank	0.357, 0.371, 0.382
"	Skin of belly white and lustrous	0.293, 0.330
Psenes cyanophrys (c)	Shiny lateral flank	0.385
Hatchet fish	Skin of silvery flank	0.216
Argyropelecus hemigymnus (d)	Operculum	0.270
A. olfersi (d)	Skin of silvery flank	0.690
"	Operculum	0.558
A. hemigymnus (d)	Skin of silvery flank	0.144, 0.218, 0.257
A. lychnus (d)	Skin of silvery flank	0.659, 0.718
Lantern fish *Diaphus theta* (d)	Silvery scales of flank	0.072
Lantern fish *Myctophum punctatum* (d)	Dorsal body wall	Negative, 0.007
"	Silvery flank	0.097
"	Lateral body wall, skin plus peritoneum	0.235
	Operculum	0.136
Pinfish *Lagodon rhomboides* (a)	Silvery skin of lateral flank	0.333, 0.340, 0.341, 0.382, 0.444, 0.550, 0.572
Coho salmon smolt	Dark dorsum	0.129
Oncorhynchus kisutch (a)	Silvery flank (middle of flank below dorsal fin)	0.285
"	Skin of silvery flank	0.251, 0.285, 0.330, 0.505, 0.623
"	Silvery operculum (outer plus inner surfaces)	0.630
	Silvery peritoneum	Present, 0.063
Dasyatis sabina (a)	Skin, upper surface of wing	0.013
"	Skin of back	0.030, 0.034
Hydrolagus colliei (a)	Shiny lateral flank	0.179, 0.192, 0.203

TABLE 1 (Cont.)

Amounts of Guanine in Fish Tissues Expressed as Weight of Guanine in a Unit Surface-Area

Fish	Preparation	Guanine mg per cm²
Stomias sp. (d)	Skin of lateral body wall	0.025, 0.034, 0.054
Cyclothone sp. (d)	Flank	Negative
Poromitra crassiceps (Melamphaes rugosus) (d)	Flank	Trace
Chauliodus sp. (d)	Operculum	0.006, 0.016
"	Skin of dorsal flank	Negative
"	Skin of lateral silvery flank	Negative, 0.007
Synaphobranchus sp. (e)	Skin, lower flank	Negative
Parabassogigas crassus (e)	Skin of lateral and lower flank	Negative
Raia richardsoni (b)	Skin of lower surface	Negative
Centrophorus sp. (b)	Skin of ventro-lateral flank	Negative, 0.012
Centroscymnus coelolepis (b)	Skin	Trace
Hydrolagus affinis (b)	Lower flank	Negative
Section 2. Irides		
Trichiurus lepturus (a)	Iris	Present
Dasyatis sabina (a)	Iris	Present
D. sayi (a)	Iris	0.177
Section 3. Chorioids having tapeta lucida.		
Ratfish *Hydrolagus colliei* (a)	Chorioid, unspecified	0.313, 0.314
"	Dorsal chorioid	0.135
"	Dorsal third of chorioid	0.112
"	Central region above optic nerve	0.267, 0.308
"	Ventral fundus, below optic nerve	0.044
"	Ventral region	0.014
H. affinis (b)	Chorioid	0.164, 0.324
Centroscymnus coelolepis (b)	Chorioid	0.118, 0.188, 0.196, 0.209, 0.251, 0.321, 0.332
Etmopterus princeps (b)	Chorioid	0.324
Stingaree *Dasyatis sabina* (a)	Chorioid	0.106, 0.125, 0.166, 0.167, 0.505, 0.528, 0.700
"	Dorsal region	0.255
Say's stingray *Dasyatis sayi* (a)	Dorsal black area above tapetum	Trace
"	Dorsal region	0.946
"	Dorsal third, above optic nerve	0.846
"	Ventral field below optic nerve, black	0.498
Breviraja pallida (b)	Lateral region	0.187
Electric ray *Narcine brasiliensis* (a)		Present

(a) neritic, (b) bathybenthopelagic, (c) epipelagic, oceanic, (d) mesopelagic, (e) bathypelagic.

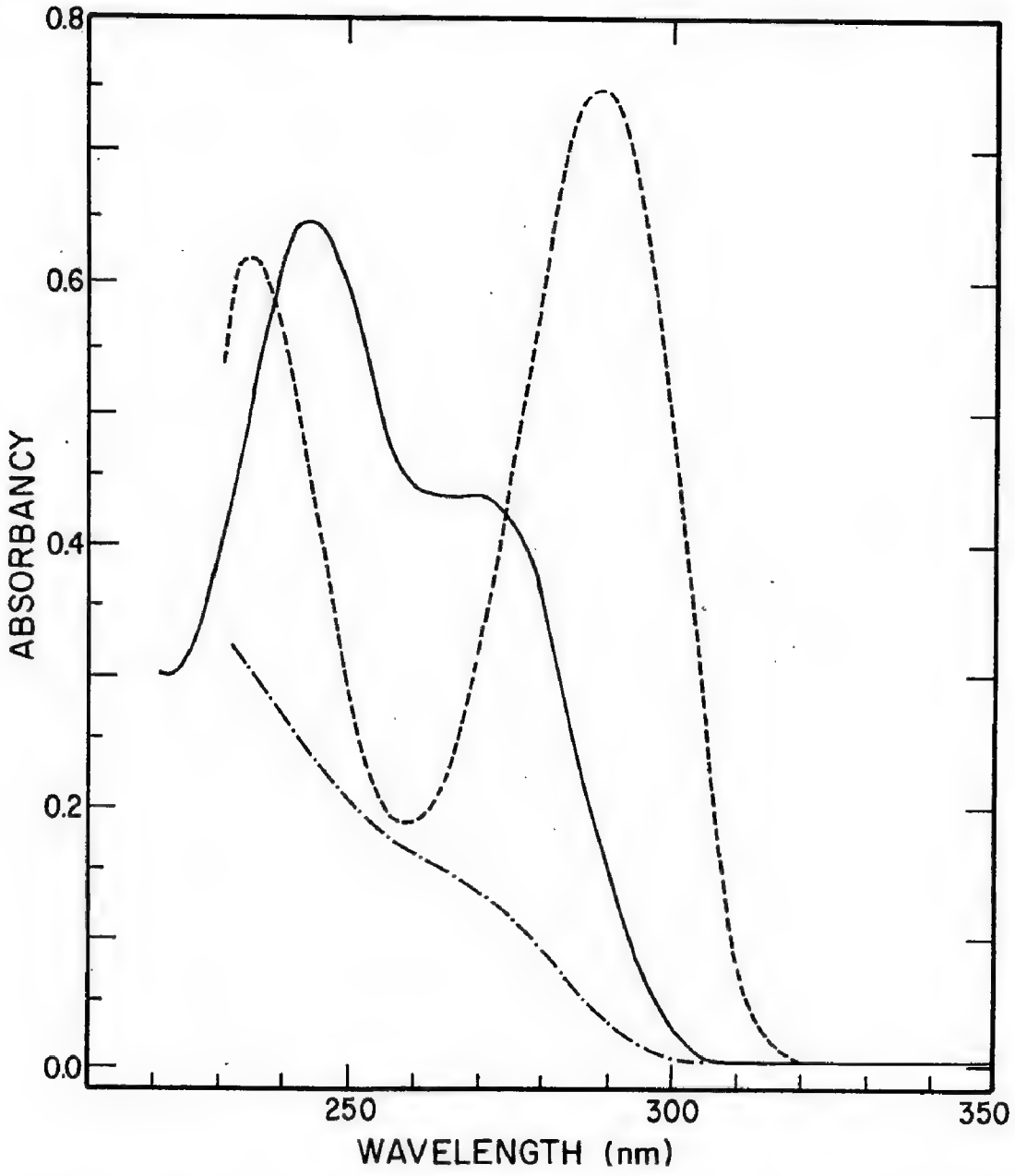

FIG. 2. Absorption spectra of an extract of clean crystals from the chorioid (tapetum lucidum) of the stingaree. Continuous line (—), crystals in Tris buffer, pH 8. Broken line (– – – –), after treatment with xanthine oxidase and guanase. Broken line + peck (— · —), final curve after treatment with uricase. Equivalent quantities of enzyme were added to both specimen and reference cuvettes.

Skin

Two very silvery and common fishes of inshore waters of the Gulf have been examined, *viz.*, the ribbon or cutlass fish *Trichiurus lepturus* and the pinfish *Lagodon rhomboides*.

The ribbon fish is like burnished aluminum, the whole surface shines. A description of its optical properties is presented in a later section (p. 82). The amount of guanine per unit surface area of skin is the highest encountered, 0.35 to 0.72 mg per cm^2. The silvery layer is a superficial stratum argenteum.

The pinfish is laterally compressed and its surface is very silvery. The sides of the body bear alternating silver and yellow longitudinal stripes. Melanophores outline the posterior rims of the scales, and there is a thin stratum argenteum in the dermis beneath the scales. Guanine levels, though high, are less than in the skin of the ribbon fish, ranging from 0.33 to 0.57 mg per cm^2.

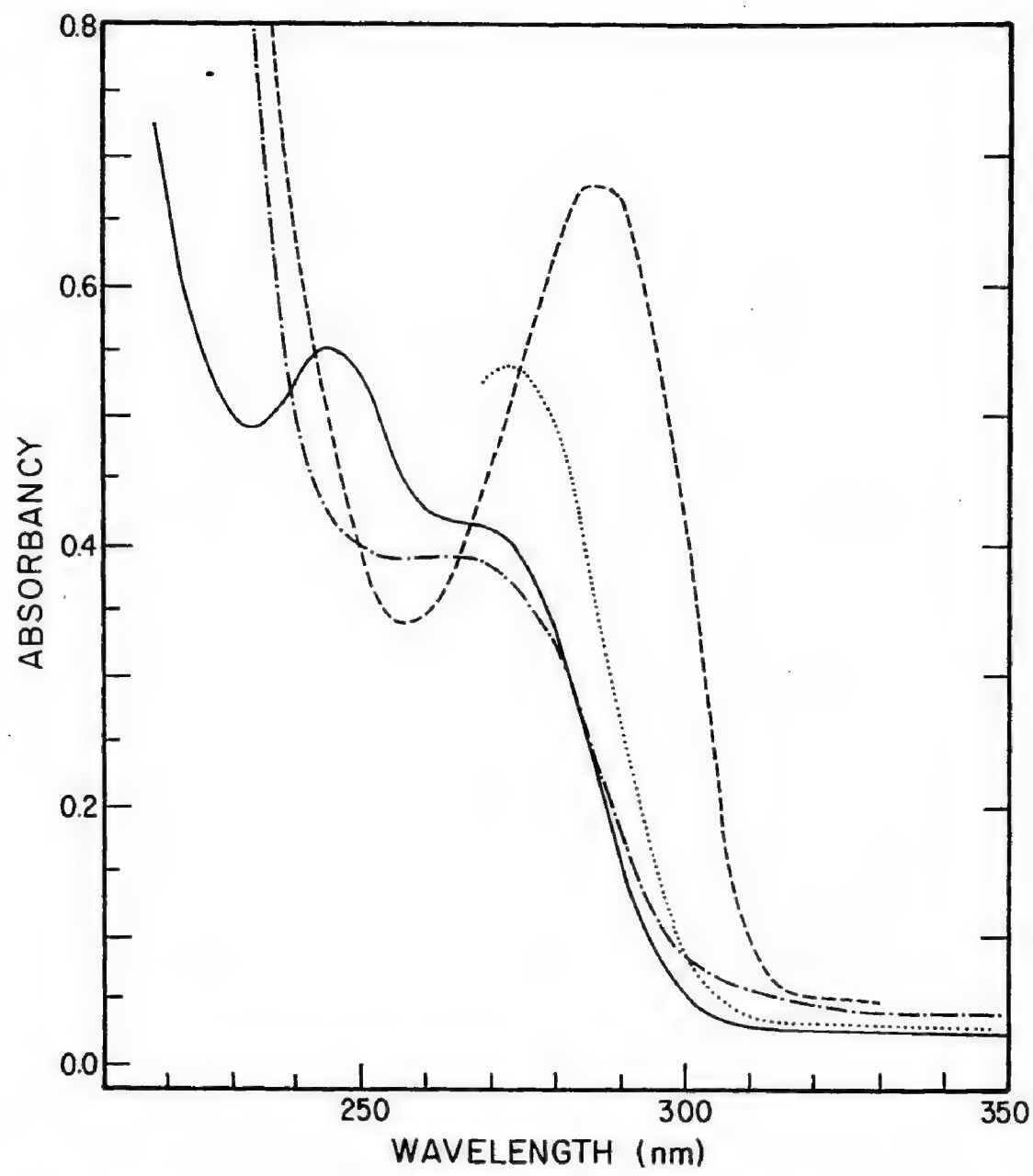

FIG. 3. Absorption spectra of an extract of the chorioid of the stingaree. Continuous line (—), crude extract. Pecked line (. . .), after addition of xanthine oxidase. Broken line (– – –), after addition of guanase, reaction complete. Broken + peck (— · —), final curve after addition of uricase.

The cigar or lizard fish is benthic; it is brownish and somewhat translucent above, and silvery or lustrous on the lower sides and belly. The flanks contain an oriented reflecting layer. There is very little guanine in the skin of the brown back, and fairly high levels in the skin of the flank and belly, *viz.*, 0.29 and 0.38 mg per cm^2.

Less silvery is the golden croaker *Micropogon undulatus*. Guanine content of skin ranges from 0.15 to 0.27 mg per cm^2.

The coho salmon is anadromous; young fish, when about to descend to the sea, become silvery and are called smolts. Specimens of smolts secured were partly silvered; they varied somewhat and some still showed parr markings through the silver sheen. Not surprisingly, there was a spread of values for guanine, from 0.25 to 0.62 mg per cm^2. Silvery salmon contain both oriented reflecting layers and strata argentea (Denton and Nicol 1966).

One epipelagic fish was examined, *Psenes cyanophrys* (from the shore at Port

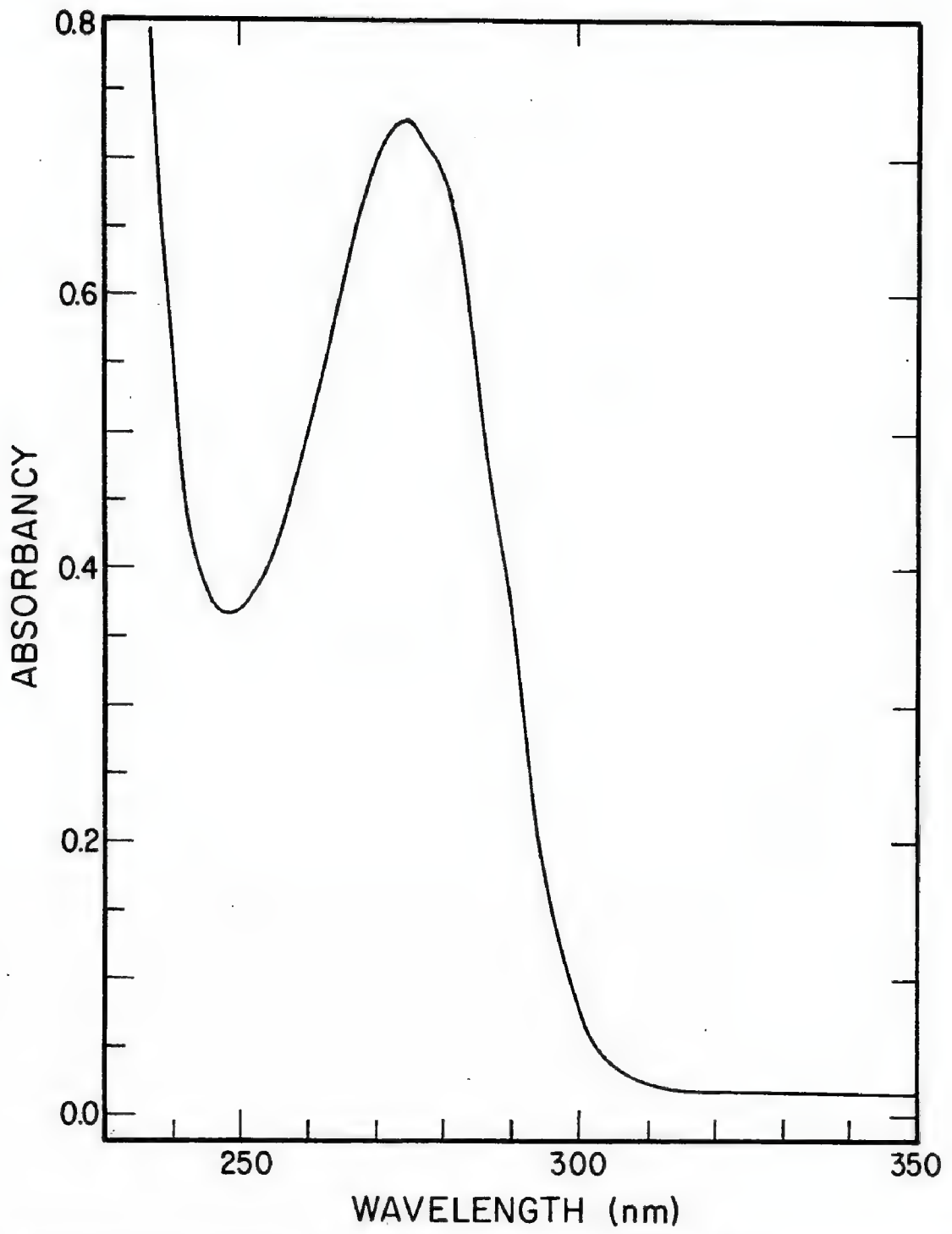

FIG. 4. Absorption spectrum of an NaOH extract of the eye of a squid *(Lolliguncula brevis)* in Tris buffer (at pH 8).

Aransas). This fish, like many epipelagic teleosts, was bluish above and had silvery flanks; the skin of the flanks contained a fairly high level of guanine, 0.39 mg per cm^2.

Silvery mesopelagic fish examined were hatchet fish (Sternoptychidae) and lantern fish (Myctophidae). Hatchet fish are black above, and have silvery flanks and photophores. There is a clear area in the posterior trunk. The silvery layer of the skin, superficial and very delicate, contains much guanine, up to 0.70 mg per cm^2 in *Argyropelecus olfersi* and *A. lychnus*. This layer is a superficial stratum argenteum.

Lantern fish execute vertical migrations (Pearcy 1964). In *Diaphus theta* and *Myctophum punctatum* there is an oriented reflecting layer beneath the scales but apparently no underlying stratum argenteum. The body wall has a bluish

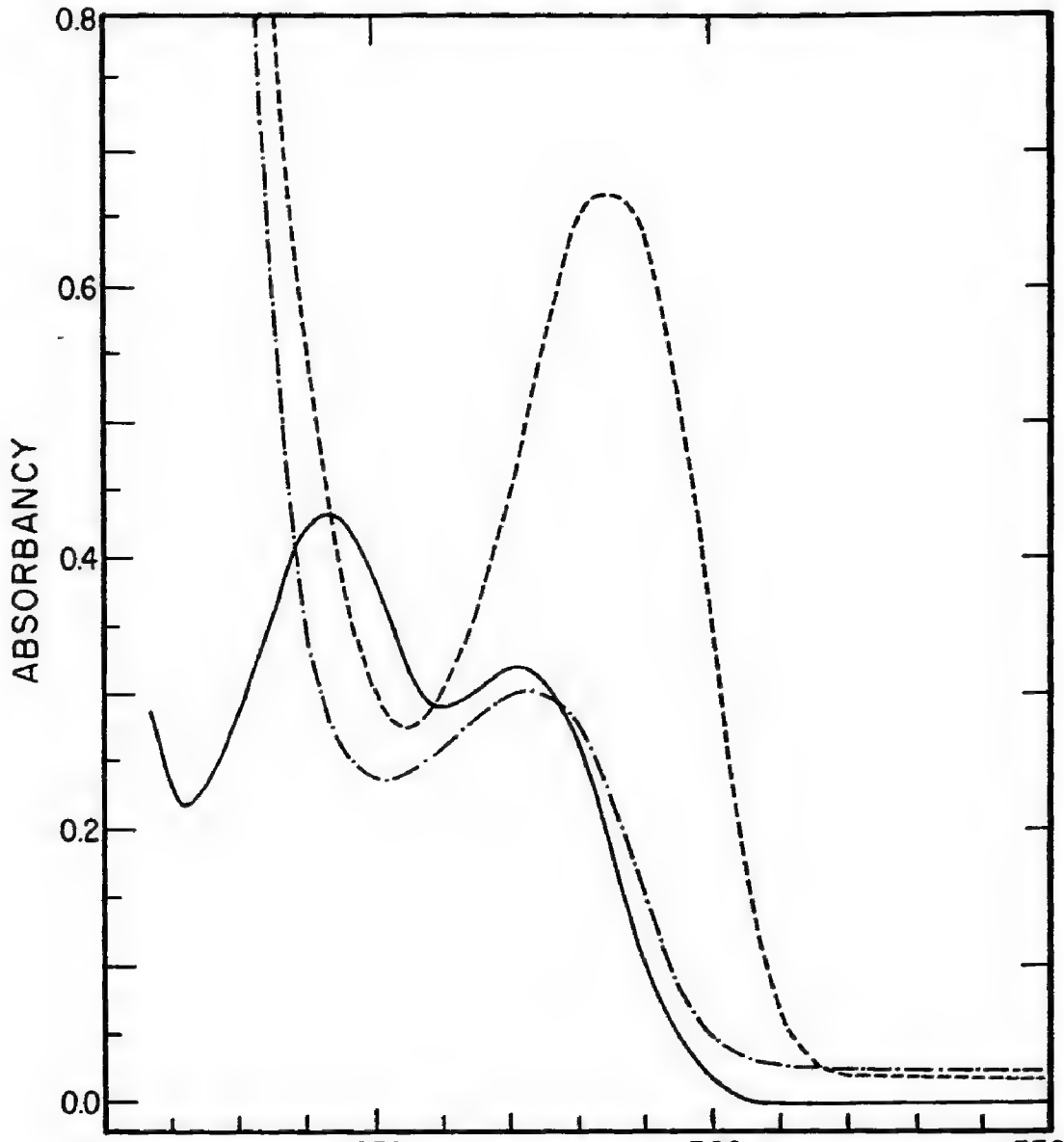

FIG. 5. Absorption spectrum of an extract of the skin of the ribbon fish *(Trichiurus lepturus).* Continuous line (—), crude extract. Broken line (– – –), after treatment with xanthine oxidase and guanase. Broken + peck (— · —), final curve after uricase.

tint, possibly owing to scattering of light. The dorsum is blue, the flanks metallic blue. The dorsal integument of *M. punctatum* contains very little guanine; what there is probably is located in a line of small glittering blue iridophores in the upper flank. The silvery scales of the flank contain 0.07 to 0.1 mg of guanine per cm^2 (*Diaphus theta* and *Myctophum punctatum*).

Of other mesopelagic fish examined, *Cyclothone* is dusky or black (the species examined) and contains no guanine. *Stomias* and *Chauliodus*, although black, have a perceptible bronze or silver sheen along the flanks. Guanine is present in small amounts, 0.01 to 0.05 mg per cm^2. *Poromitra crassiceps*, very black, contains only a trace of guanine.

The skin of a deep-sea eel *Synaphobranchus* and a brotulid *Parabassogigas*, both dark and brown, contains no free guanine.

Elasmobranchs are not usually thought of as being iridescent or of having a metallic luster. The stingaree, however, although dull brown when observed from above, has a bronze sheen when seen from the side, the light coming from above;

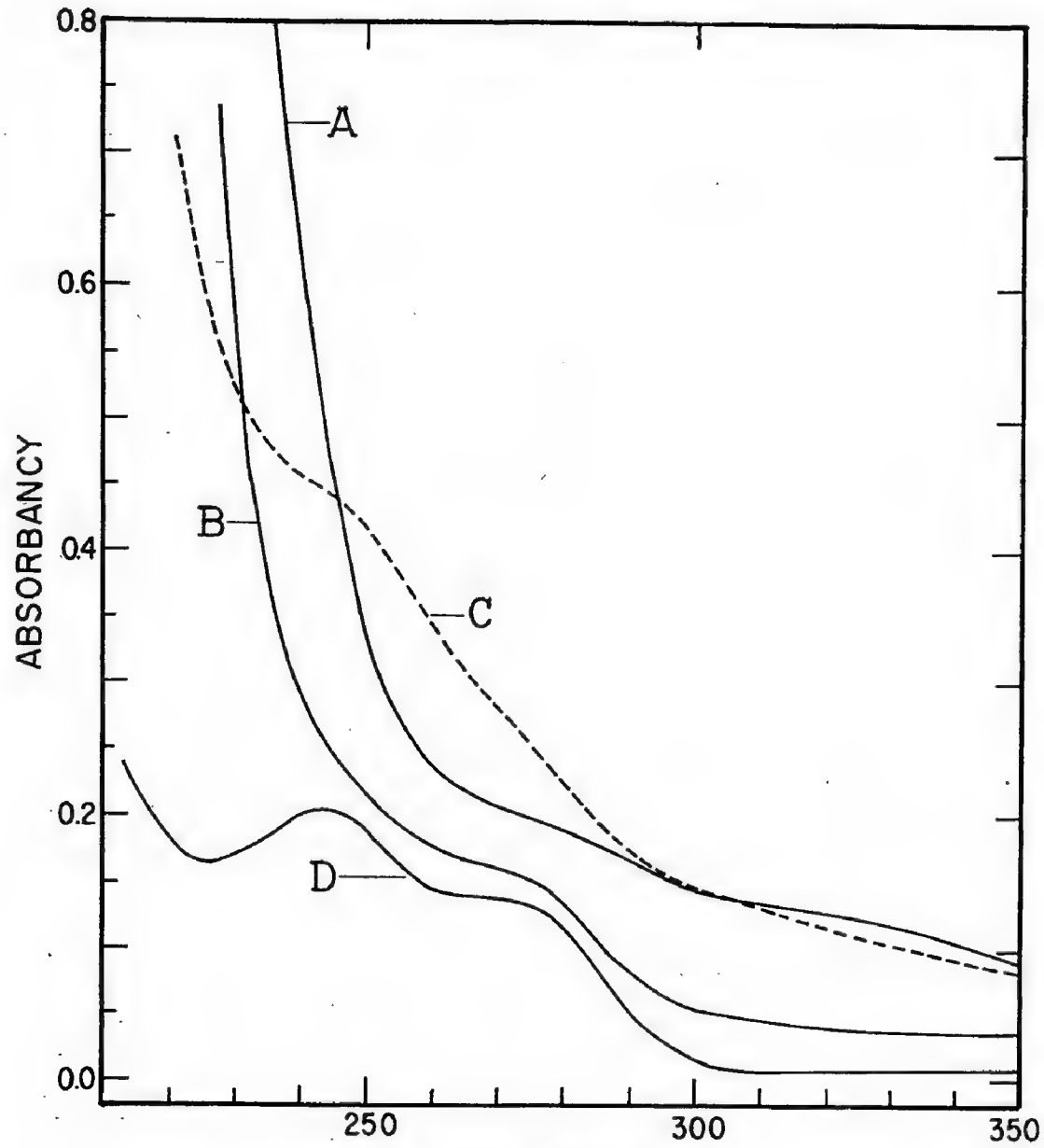

FIG. 6. Absorption spectra of the skin of various fishes and of an extract of hair (containing melanin). A, human black hair. B, dark *Cyclothone* (no guanine). C, *Myctophum punctatum*, dark dorsum (no guanine). D, *Argyropelecus hemigymnus*, silvery flank.

the sheen is like bronze-paint. The amount of guanine present is low, up to 0.03 mg per cm^2. Among deep-sea rays and squaloids examined, the skin contains traces of guanine (*Deania*) or gives negative assays. Similarly, the skin of a deep-water chimaerid *Hydrolagus affinis* lacks guanine, whereas a shallow-water species *H. colliei* is well endowed, having 0.2 mg per cm^2. The latter is silver or gold and exhibits a high degree of metallic reflection in life. *Chimaera monstrosa* and some other chimaerids are also silvery (Hardy 1959; Dean 1906) from which circumstance they appear to have acquired the name "king of the herrings".

Several additional features may be noted in Table 1 (section 1). The operculum when it is silvery contains a considerable amount of guanine; there are silvery layers on both outer and inner surfaces, *e.g.*, coho smolt. White and opalescent bellies contain much guanine, *e.g.*, lizard fish. There is guanine in the silvery peritoneum, but the amount is fairly meager (coho smolt).

Guanine occurs in the irides of three species of fishes examined, all three having silvery or other reflecting layers (Table 1, section 2).

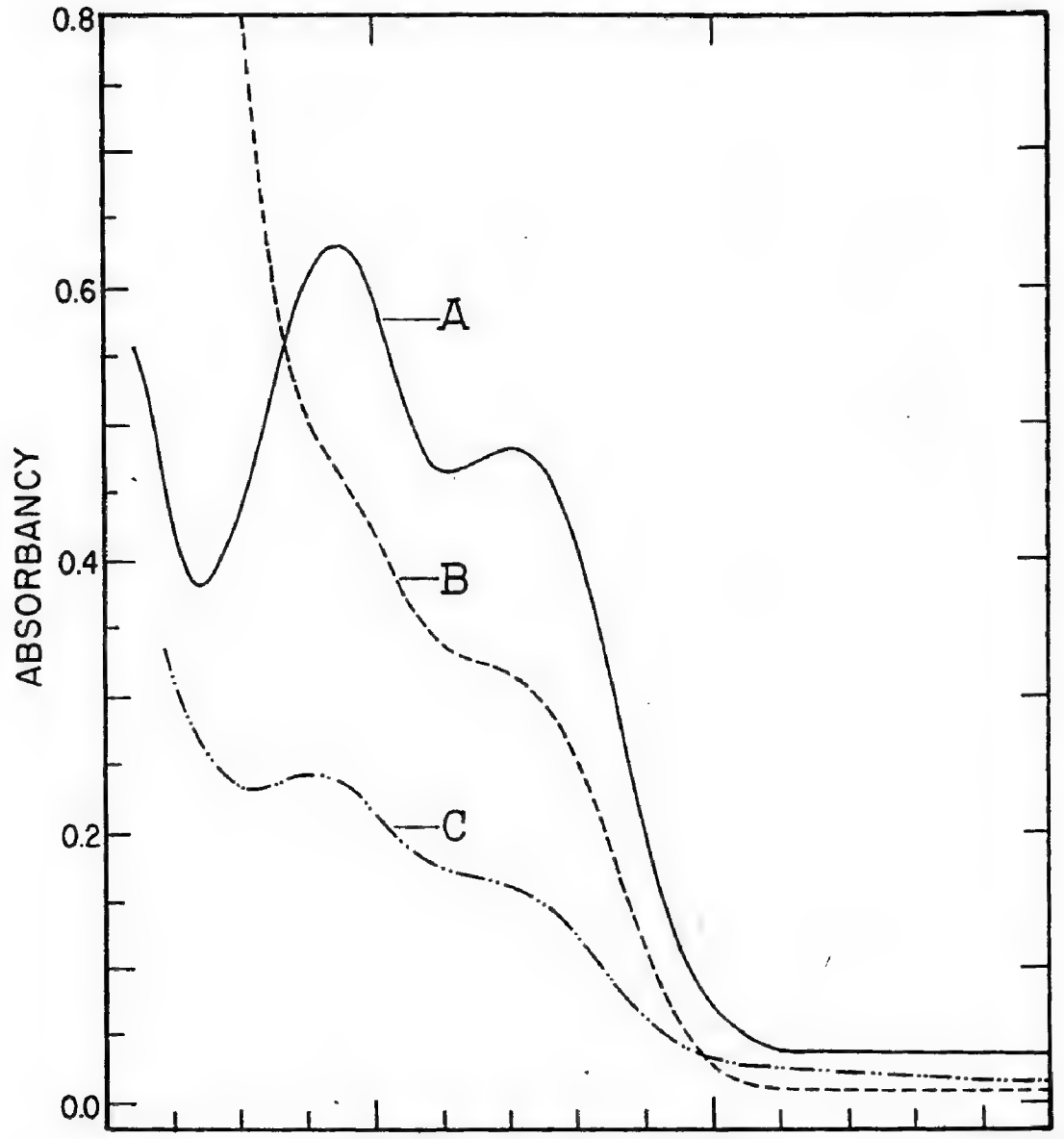

FIG. 7. Absorption spectra of crude extracts of the skins and the eye of several fishes. A, *Argyropelecus olfersi*, silvery flank. B, *Hydrolagus colliei*, silvery flank. C, *Centroscymnus coelolepis*, chorioid.

Ocular Tapeta Lucida

Tapeta lucida in the chorioids of elasmobranchs and chimaerids contain high levels of guanine. A range of values is displayed in Table 1, section 3; they mostly lie between 0.1 to 0.3 mg per cm^2 of chorioid surface. The tapeta lucida of both deep-water and shallow-water chimaerids and selachians contain guanine to an equal extent. From what we know of the fine anatomy of the eyes of these animals we can venture a few correlations. The reflecting cells of the tapetum lucidum change in orientation from the center to the periphery of the eye (Denton and Nicol 1964; Arnott and Nicol, unpublished observations). They are stacked in depth in the central fundus and arranged in single file toward the periphery. This changing arrangement may account for the regional variation of guanine content, as in the ratfish's chorioid. Also, in this animal, the ventral field of the tapetum lucidum is pigmented and dark, and the level of guanine is low. The chorioids of stingarees contain astonishingly large amounts of guanine, up to 0.95 mg per cm^2. It is now known that the tapeta lucida of these animals are organized

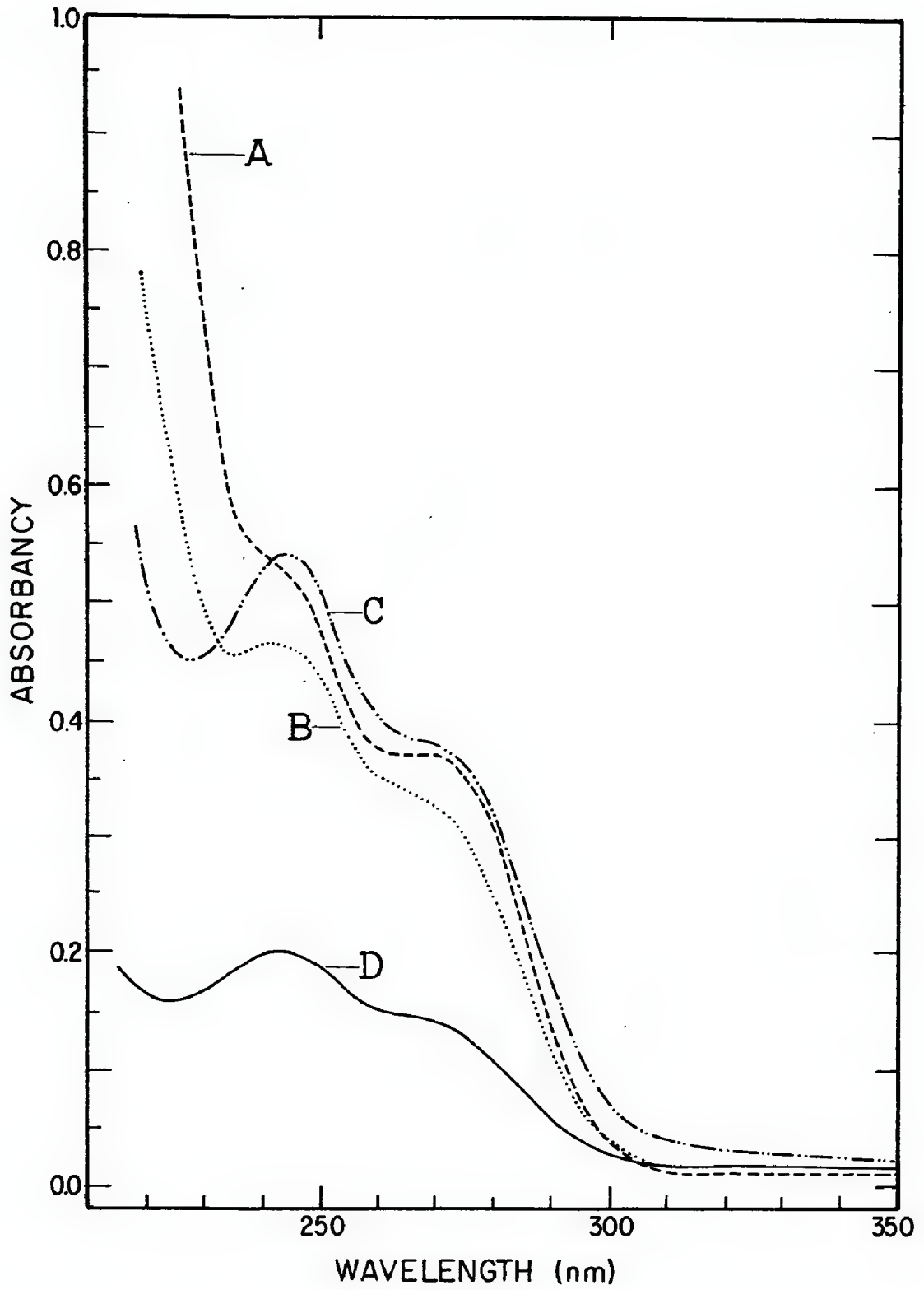

FIG. 8. Absorption spectra curves of crude extracts. A, silvery skin of pinfish. B, silvery operculum of coho smolt. C, chorioid of *Hydrolagus affinis*. D, silvery operculum of *Myctophum punctatum*.

in a novel manner, having many reflecting cells throughout the thick chorioid (Arnott and Nicol, unpublished observations). The density of reflecting cells, therefore, in a unit area of stingray chorioid is very high. The eyes of Say's stingray are larger than those of the stingaree, and it is easier to obtain samples of different areas of chorioid from them. Results of assay reveal that the dorsal and central regions of the chorioid of Say's stingray contain almost twice as much guanine as the ventral chorioid, which tends to be pigmented.

Examination of Cleaned Crystals

Two methods were employed for cleaning crystals. The clean crystals were then investigated by enzyme-assay and paper-chromatography.

Method 1. Silvery material (from the surface of the ribbon fish) was dried at 70 C and then ground up with a little dichloroethane. A column of graded densities was made with bromoform, chloroform, and dichloroethane. The material, in dichloroethane, was added to the top of the column. In two hours, shiny particles (the crystals) advanced down the tube, whereas the tissue fragments remained above. The layer containing the crystals was collected and centrifuged; the centrifugate was washed with acetone twice, then with petroleum ether and water. Finally the material was taken up in a little 0.1 N sodium hydroxide.

Method 2. Silvery material (again from the ribbon fish) was stored frozen. On thawing, the material was suspended in 0.5 M phosphate buffer, a little trypsin and peptidase were added, and the container left on a shaker overnight at room temperature (24 C) (a drop of toluol was added to halt bacterial action). Much of the crystalline material aggregated in dense clumps. The clumps were picked up with forceps, washed with water and divided into two lots. One lot was dissolved in a little 0.1 N sodium hydroxide. The other was centrifuged, washed with acetone and petroleum ether, dried, and then dissolved in 0.1 N NaOH.

An aliquot of the digested suspension was centrifuged and the centrifugate washed with water. It was taken up in water and added to the top of a graded density column. Overnight three zones developed, the lowermost contained finely dispersed shiny particles, the next clumps of crystals, a lower white zone contained some crystals, and a clear layer formed at top. The bottom zone was drawn off, centrifuged, and the centrifugate washed with water and dissolved in 0.1 N sodium hydroxide.

These several methods yielded preparations of fairly clean crystals. The absorbance spectra were very similar to that of pure guanine. Addition of xanthine oxidase and guanase caused a shift of the absorbance maximum to 290 nm, evidence for conversion to uric acid, and the addition of uricase abolished the peak at 290 nm (Figs. 9 and 10).

Partially cleaned crystals from the tapetum lucidum of the stingaree were secured in this manner. Chorioids, freed of retina, were frozen, thawed, and broken up vigorously in water. The mix was passed through several thicknesses of cheese cloth, centrifuged, washed, and suspended in 0.5 M phosphate buffer at pH 7.1. Trypsin, peptidase, and a drop of toluol were added; the suspension was left to digest overnight. It was then centrifuged, washed, and added to the top of a tube containing a saturated solution of sucrose. The tube was centrifuged, the supernatant discarded; the procedure was repeated, the centrifugate washed and then dissolved in 0.1 N sodium hydroxide. Most tissue elements and melanin particles were removed by this treatment. The absorbance curve of the dissolved material is like that of guanine, except that the pronounced trough at 260 nm is wanting, where residual melanin may be responsible for raising the absorbance.

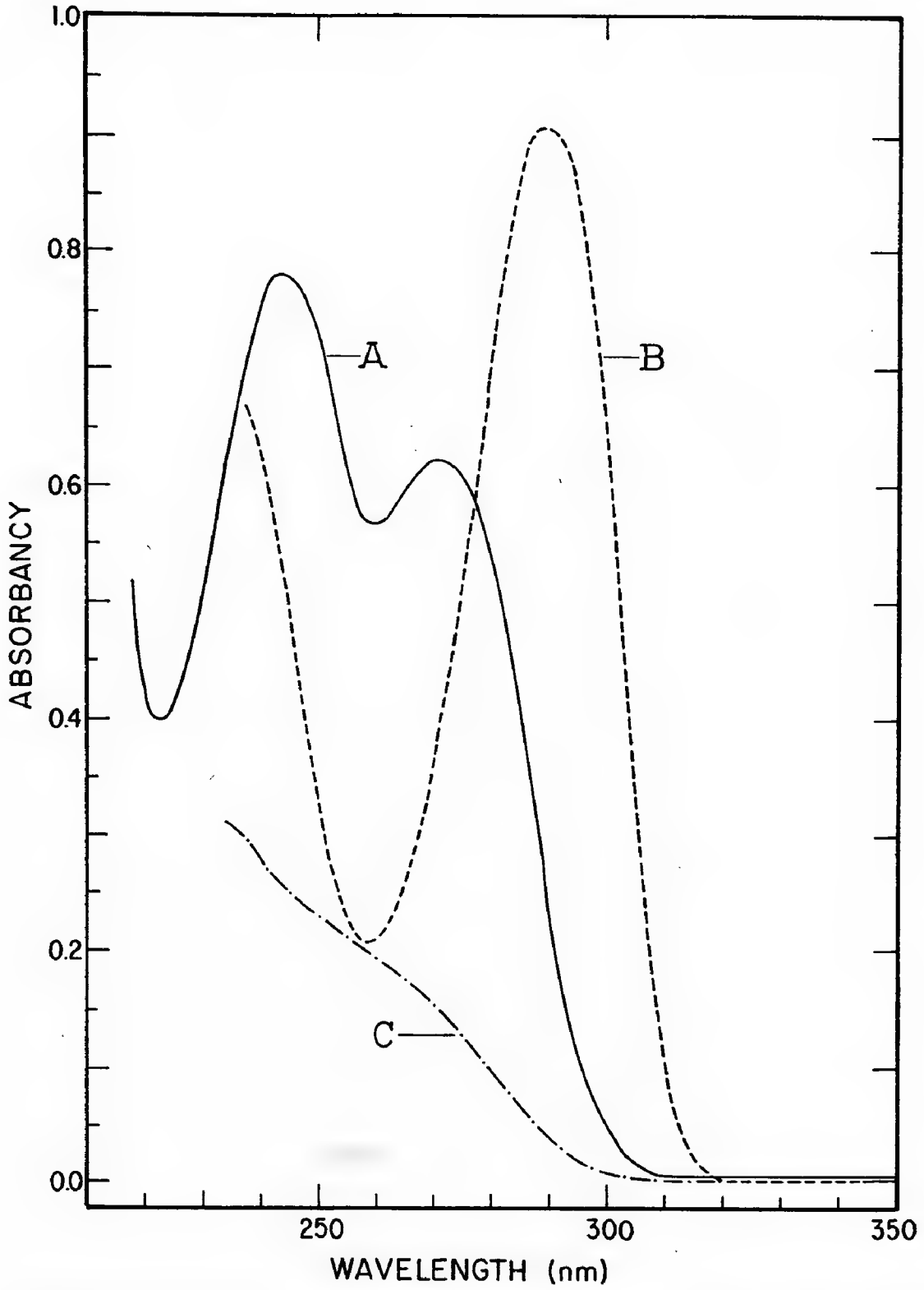

FIG. 9. Absorption spectrum of cleaned crystals from the skin of the ribbon fish. A, extract of crystals. B, addition of xanthine oxidase and guanase, conversion to uric acid. C, final curve after addition of uricase. Enzymes added to specimen and reference cuvettes.

Enzyme assay showed the usual shift to 290 nm (uric acid) and disappearance of that peak on adding uricase (Figs. 2 and 10).

Squid

Fresh squid (*Lolliguncula brevis*) trawled from the Gulf, were examined for silvery surfaces. Two such surfaces are present, one on the oculus, the other on the ink-sac.

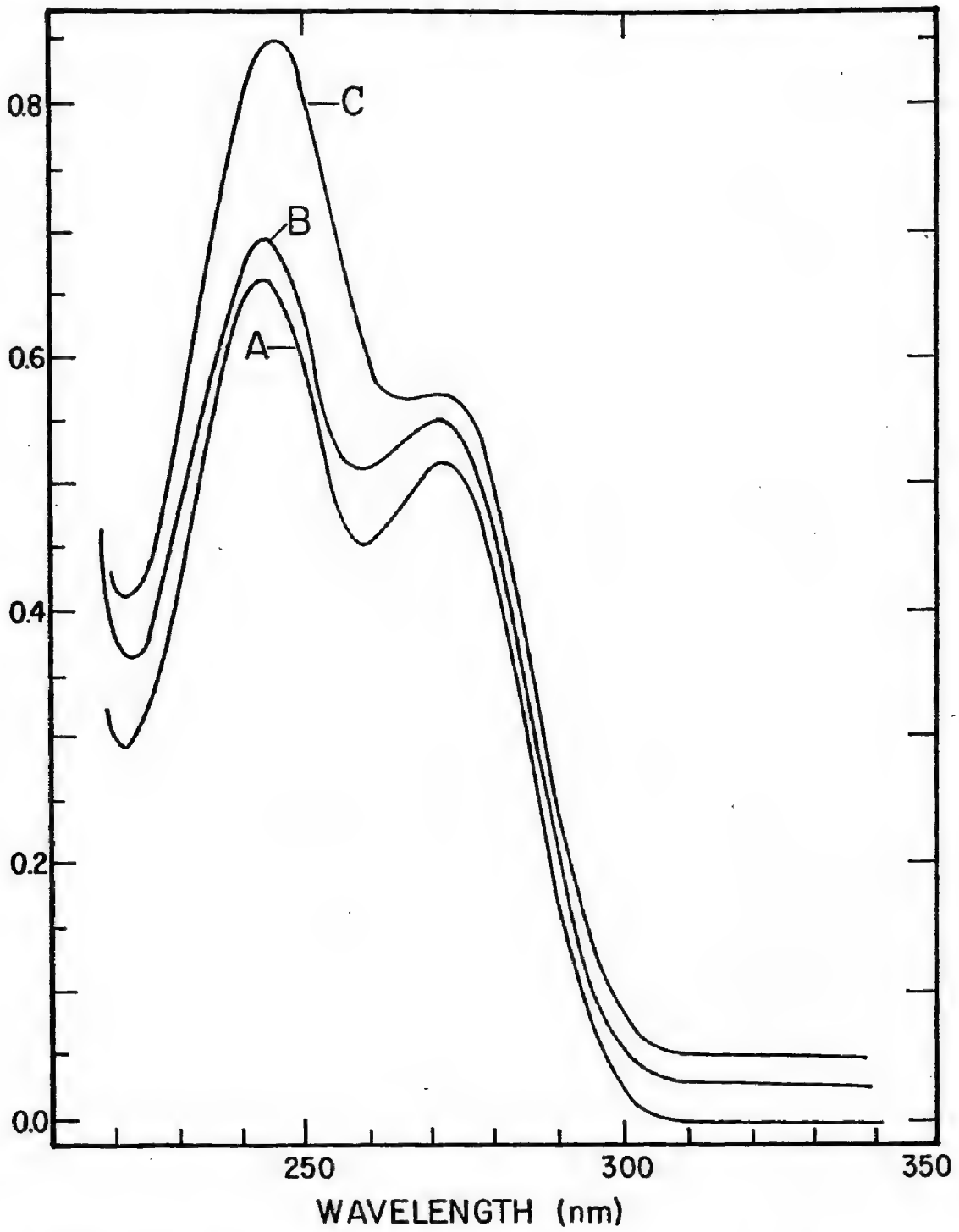

FIG. 10. Absorption spectra of solutions of pure guanine (curve A), of cleaned crystals of ribbon fish (curve B) and of cleaned crystals of the chorioid of the stingaree (curve C). The three curves are normalized at 572 nm (O.D. 0.52). For clarity of presentation, curves B and C are displaced upwards 0.025 and 0.050, respectively.

The exterior of the eyeball and of the iris is silvery and very reflecting, except for a stripe on top; the latter is black with a greenish overlay. An operculum overhanging the upper part of the pupil is very silvery. An external covering on the ink sac is very silvery and conceals the black contents. These silvery surfaces are iridescent, and the colour changes as the angle of viewing alters, from reddish at normal incidence to green towards grazing incidence.

A piece of the silvery covering of the eye was teased into distilled water. By transmitted light many fine needles could be seen, pointed at both ends, some fairly straight, others curved or crinkled. In fragments of tissue and at torn edges the needles were seen to be arranged in bundles, parallel to each other. Needles appear to be circular in section. Tissues were steeped in 0.1 N NaOH and enzyme-

assayed for guanine. None was found. The absorption curve has a single peak at 272 nm (Fig. 4).

Fluorescence and Chromatography

The fresh (surviving) chorioid of the stingaree, from which the retina has been removed, fluoresces yellow-green in long wavelength UV (365 nm). When the surface of the chorioid is scraped gently, the scraped area loses its fluorescence. Fluorescence possibly occurs in the pigment-epithelium.

Chorioids of stingarees were collected, the retinae removed, and the chorioids pooled and stored frozen.

The material was extracted with 50% acetone. The extract was examined by ascending paper-chromatography, with a solvent-system consisting of n-propanol and 3% ammonium hydroxide, 2/1 v/v, on Whatman 3 MM paper. Chromatographed as described, the extract gave at least four distinct fluorescent spots, *viz*:

Spot 1, yellow-green fluorescent
Spot 2, blue fluorescent
Spot 3, purple fluorescent
Spot 4, purple fluorescent

The residual material was treated with 0.1 N NaOH, which effected a further degree of extraction. The same spots were evident as those described in the preceding paragraph, and also two quenching spots (A and B) (Fig. 11).

Reference Spot 3, purple fluorescent. A strip chromatogram was made of the acetone extract, with propanol-ammonia as the solvent system. The region occupied by band 3 was eluted with water, and the eluate was co-chromatographed with isoxanthopterin in four solvents, *viz*., propanolammonia, 4% sodium citrate, 5% acetic acid and butanol-acetic (n-butanol, 50% acetic acid, 2/1 v/v). In all four solvents the eluate of band 3 was the same as a sample of authentic isoxanthopterin, in Rf values and in the color of fluorescence. The chromatography provides strong presumptive evidence that the material from the chorioid contains isoxanthopterin.

Spot 1 is of particular interest because it is a major component of the total fluorescent material. It is yellow-green fluorescent and visibly yellow. Co-chromatograms were run that showed that it is not one of a number of pteridines, i.e. not xanthopterin sulfate, xanthopterin, erythropterin, nor FMN, riboflavin, compound G (Van Baalen and Forrest 1959) nor lumazine. Its resolution awaits further study.

Scrapings of the silvery skin of ribbon fish were collected and pooled and stored frozen. After being thawed, the material was digested with trypsin and peptidase; the flask containing the digestion mixture was placed on a shaker and left overnight (24 C). Crystals aggregated in clumps, which were picked up with forceps, washed with water and dissolved in 0.1 N NaOH. The material was chromatographed in n-propanol; 3% ammonia (2:1) as solvent. The presence of guanine was confirmed by paper chromatography. Additionally, two other quenching substances were present, probably hypoxanthine and uric acid. The chromatograms also showed a yellow-green fluorescent band. Rf values are:

	Extract	Known
Spot 1	yellow-green fluorescent 0.09	
Spot 2	quenching 0.20	uric acid 0.20
Spot 3	quenching 0.30	guanine 0.30
Spot 4	quenching 0.48	hypoxanthine 0.47

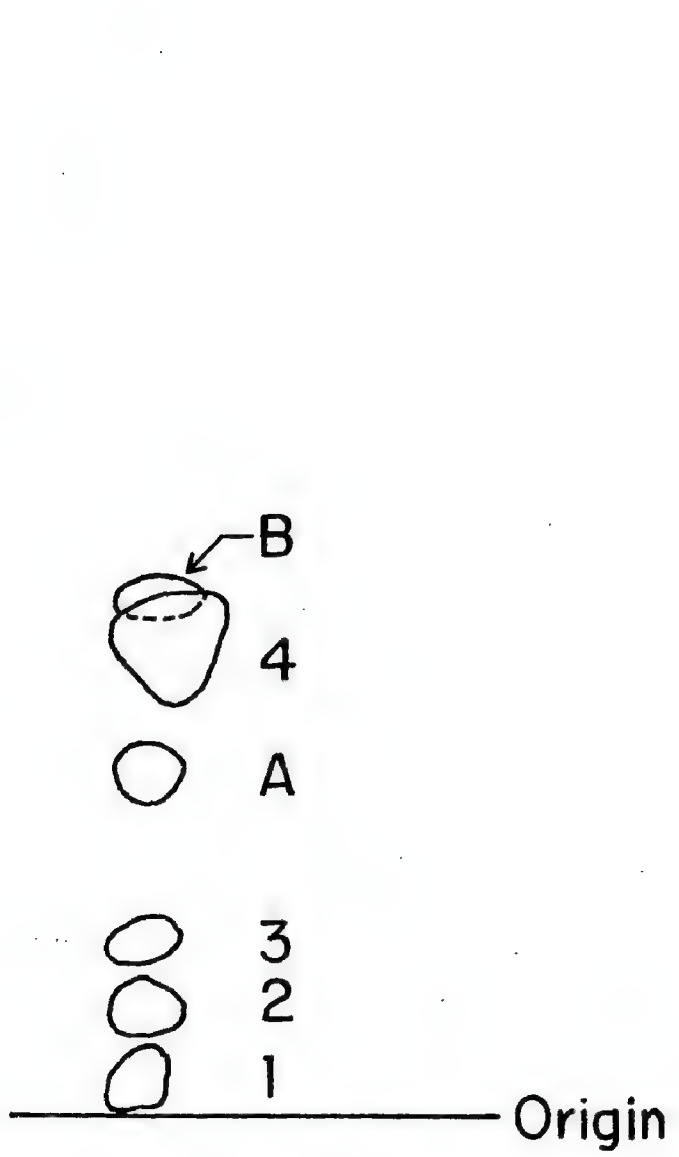

FIG. 11. Chromatogram of an extract (in NaOH) of the chorioid of the stingaree (see text for explanation).

The Silvery Surface of the Ribbon Fish

The ribbon fish was one of the fishes examined most intensively because of its splendid stratum argenteum. It was desirable, therefore, to study the optical properties of the reflecting layer.

Examined microscopically the silvery surface of the ribbon fish is covered with fine streaks or needle-like stripes. These are arranged longitudinally (antero-

posteriorly) at all levels. The streaks correspond to fine rachidial crystals. The overall microscopic appearance is as polished silver or aluminum, but microscopically streaks exhibit a medley of colours, red, yellow, green, blue and purple (these are the colours of superposed crystals, which will be referred to as reflecting platelets). The combination of colours gives rise to the white coloration of the fish's surface. The skin contains many dispersed melanophores except for a a narrow region immediately above and below the lateral line.

A band of aluminum foil was wrapped around a fish, which was laid on a gimbals table and examined microscopically by vertical illumination. The table was moved about the antero-posterior and dorso-ventral axis of the fish until maximal brightness was observed, and the angles were noted. A series of readings was made in a dorso-ventral line around one side of the fish. Skin and foil at each focus were brightest at the same angles of rotation, indicating that reflecting platelets and foil had the same orientation. Angles to verticals parallel to the median dorso-ventral axis of the fish were plotted on a cross-section of a fish; the reflecting platelets were found to be parallel to the surface of the body.

Pieces of fish were mounted in a V-shaped chamber, in which they could be illuminated diffusely from one side. The chamber was movable about a pivot at the apex of the V. The fish was examined microscopically from above. Inspection showed that the surface of the fish is silvery everywhere except for a narrow dark stripe along the center of the back. The upper flanks, towards the dorsal edge of the body, appear dusky and dark when viewed from above, and silvery when viewed from the sides and below. The sides of the body, when viewed at near normal incidence, lack silverness and appear to some degree translucent. Viewed at angles away from normal incidence, the surface is silvery except at steep angles, towards grazing incidence, when some blue colour creeps in.

A cross-section of ribbon fish is shown in Figure 12. The body is particularly slender and laterally compressed. It also differs considerably from the usual shape of a fish in being narrow and pointed above and broadened below. In these respects it is in strong contrast to the herring, another silvery fish which has been much studied (Denton and Nicol 1965). Because of the compressed shape of the ribbon fish the reflecting platelets, over much of the lateral surface, are oriented nearly parallel to the median longitudinal vertical plane of the fish. In the upper third of the body they are tilted slightly upwards, below the lateral line, downwards.

These various arrangements help to camouflage the fish in its special environment. The waters where it lives are especially turbid, the light becomes much scattered, and the distribution of light becomes symmetrical a very short distance below the surface.

The dark or dusky upper surface, viewed from above, blends into the dimly illuminated background. The silvery side reflects light coming from one side of the fish that is about as bright as the light coming from the same angle of tilt on the other side of the fish (*see* Fig. 12). The reflecting platelets, however, are not truly vertical in all regions; some of the consequences of having platelets tilted away from the vertical have been discussed for the special case of the herring (Denton and Nicol 1965). Although good reflectors, the platelets reflect only

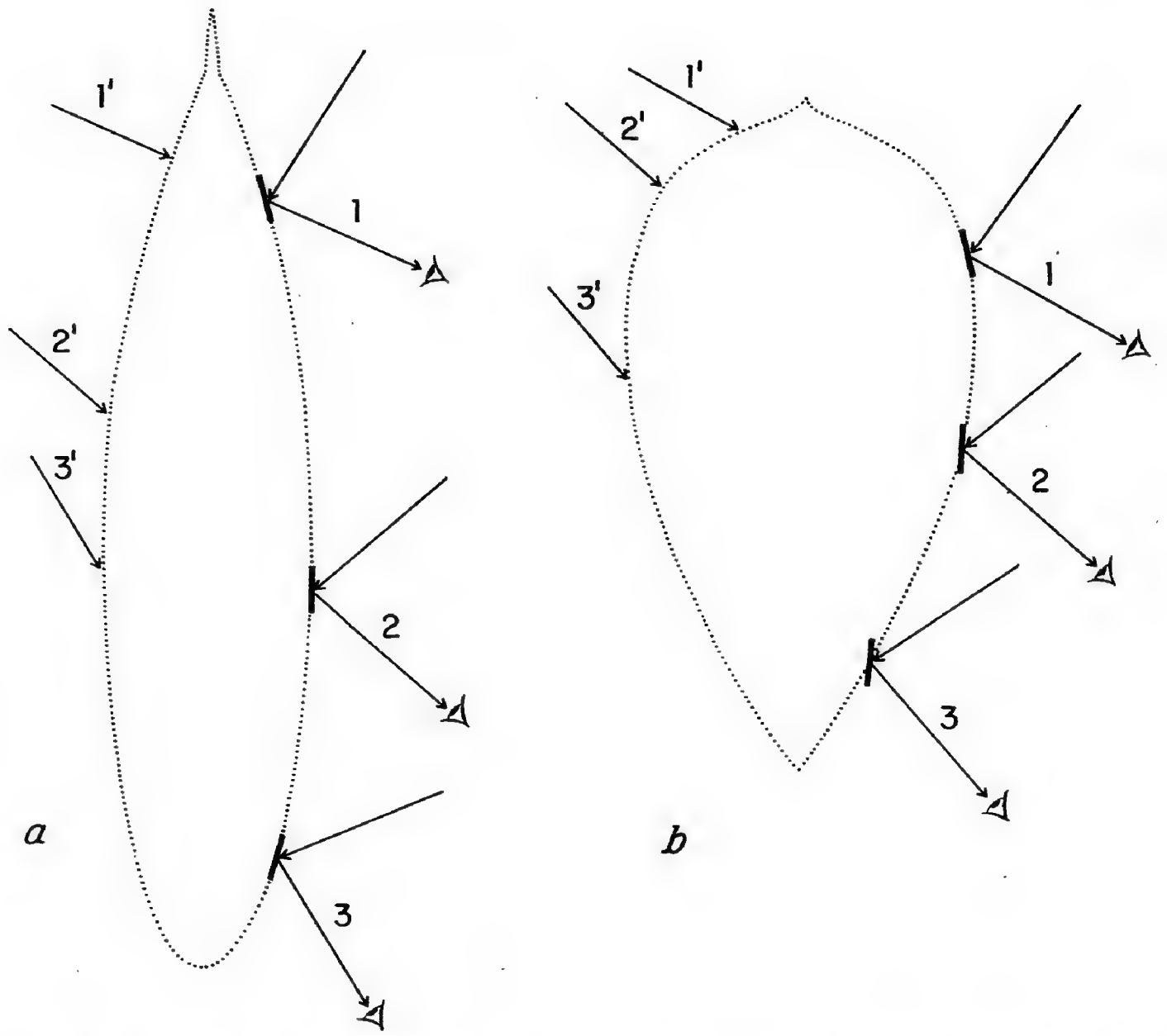

FIG. 12. Comparison of a ribbon fish (a) and a herring (b), both shown in cross-section. The illustrations show the outlines of the fish, the orientations of the reflecting platelets, and how light is reflected to an observer below. Arrow 1 shows light, originating above, that is reflected to an observer below, and this light is compared with light (represented by arrow 1′) coming from the other side of the fish, and so on. (Fig. 12b is based on Denton and Nicol 1965).

about 85% of the incident light (Denton and Nicol 1966). Tilted upwards, the platelets reflect light from a direction nearer the vertical and brighter than they would do if they were truly vertical; consequently, they can match more closely the brightness of the field on either side of the fish (Fig. 12). It is common for platelets on the lower surfaces of fishes to incline downwards, thereby reflecting downwards whatever light reaches those surfaces inevitably shadowed from above.

Melanophores, by their contraction and expansion, provide a mechanism of regulating the degree of reflection.

The whole reflecting surface of the ribbon fish is a remarkable parallel to that of the herring, but one in which the overall effect is achieved in a different way (Fig. 12). The reflecting platelets are oriented generally in the same directions with reference to the vertical as they are in the herring, but the orientation in the ribbon fish depends upon the shape of the body, whereas in the herring the platelets

are tilted and oriented independently of the body surface with reference to the vertical. The ribbon fish, utilizing a stratum argenteum for the purpose, employs closely packed, similarly aligned, rachidial crystals; the herring, broad crystals of changing orientation. The silverness of the ribbon fish is produced by the blending of a mosaic work of small coloured areas; that of the herring, by superposition of three differently coloured layers. The compressed, dorsally peaked shape of the ribbon fish is one that allows the crystalline structure of the stratum argenteum to be utilized for effective camouflage; but it is probably one that limits swimming efficiency.

DISCUSSION

Iridescent surfaces of fishes owe their appearance to constructive interference of light reflected from superposed thin lamellae of guanine crystals (Arnott and Nicol unpublished observations). Such surfaces generally exhibit a high degree of reflectivity (Denton and Nicol 1966), owing to the additive effect of reflections from regularly stacked lamellae (Heavens 1960).

In many fishes the skin has a stratum argenteum. This is a shiny or silvery layer that contains elongate, narrow, crystals of guanine (Nakata and Matuzawa 1955; Denton and Nicol 1965). The stratum argenteum sometimes lies underneath the oriented reflecting layer (*e.g.*, in the salmon and herring) (Denton and Nicol 1966), or it may be the only silvery layer present (*e.g.*, in the ribbon fish where, as a thin superficial sheet, it envelopes nearly the whole body) (this study).

In the present study fishes in variety from different habitats have been sampled, fishes that exhibit varying degrees of silver or none at all. The amount of guanine present in tissues was measured and expressed in terms of unit surface area because this form of reference seems the most significant one where the guanine is concerned with reflection of light.

Generally, when guanine is present in silvery layers of teleosts of neritic and upper pelagic waters, the amount present (per unit area) is high, one species compared with another. It is as great, for example, in the skin of the estuarine croaker as it is in the mesopelagic hatchet fish (Table 1).

Amounts of guanine in elasmobranch and holocephalan tapeta lucida are generally about the same as in the skin of silvery teleosts. Also, amounts in chorioids of shallow-water and deep-sea species exhibit no regular differences.

To achieve good reflectivity the surface of the tissue must be covered completely with platelets of guanine stacked in depth, some ten or more (Heavens 1960). Generally, it would seem that when an animal is presented with the problem of reflecting light to achieve good camouflage, it must needs have a good mirror, which depends upon a minimal layering of platelets of guanine. When downwelling light is above visual threshold, the mirror must be efficient to enable the fish to match background light and be invisible, no matter what the intensity of the illumination is, *i.e.*, the need for a good mirror is independent of intensity, it is concerned with the distribution of illumination.

Not all the results lend themselves to such facile interpretations, however. There is no ready explanation why *Cyclothone*, which ranges in hue from par-

tially transparent to black (this is a species-trend with increasing depth), lack the silver that other fishes having the same depth-distribution possess. On the other hand, there is remarkably little known about the habits of *Cyclothone* and of silvery hatchet fish, whether or not they migrate, and their depth-distribution relative to light-penetration and intensity. Neither is an explanation immediately available for the faint but distinct bronze and silver sheens on the flanks of *Chauliodus* and *Stomias*, both oceanic mesopelagic fishes.

Teleosts and chondrichthians from deep waters have no deposit of guanine in the skin. Two species of closely related chimaerids form an interesting contrast in this respect, one, *Hydrolagus affinis* from deep water, having a dull integument; the other, *H. colliei* from shallow water, shining like burnished gold. In very deep waters the light is entirely luminescence and it is randomly distributed.

Tapeta lucida function under dim light conditions, in twilight, at night, and in the darkness of the ocean depths where the illumination is weak bioluminescence. Guanine levels are consistently high in tapeta lucida of cartilaginous fishes from all environments; they show some interesting features, nevertheless. In some instances when samples were secured from several regions of the eye, it is obvious that levels of guanine are greatest in the central fundus. The tapeta lucida of some neritic species are brightest in the center, and pigmented progressively towards the periphery, especially in the ventral field. Correlated with this trend is a high concentration of guanine in the central fundus and a diminution of guanine-levels away from the central fundus, especially in the more heavily pigmented ventral tapetum.

Some special features are noted. The presence of guanine in silvery irides and in the peritoneum of various fishes is confirmed. The level of guanine in the peritoneum of the coho smolt is only 1/10 to 1/4 that in the silvery flanks. The white belly of the cigar fish (a largely diffusing surface) contains almost as much guanine as the silvery flanks. Small but significant levels of guanine occur in the skin of the upper surface of the stingaree, which has a definite bronze sheen.

Experiments involving morphological colour-changes have been carried out with greenfish (*Girella nigricans*), during which they were kept for long periods on backgrounds ranging from black to white in albedo. On light-backgrounds the amount of guanine increased; on dark, it decreased (Sumner 1944). Although it has been established that guanine is laid down or resorbed under increased or reduced light-conditions, it is conjectural what tissues or cells are involved—oriented reflecting cells, stratum argenteum or iridocytes. Similarly, during the silvering of parr, the changes in the several tissues that contain the reflecting cells are still to be unravelled. The levels of guanine found in the present study are reasonably close to those reported by others, using analytic methods (Hitchings and Falco 1944; Sumner 1944; Best and Nicol 1967).

Masses of crystals (from stingaree chorioid and ribbon fish skin), isolated and cleaned, have been shown to be guanine. There is a small amount of probably adventitious material associated with the crystals obtained from the ribbon fish, probably hypoxanthine and uric acid, as well as a yellow-green fluorescent material. Guanine, hypoxanthine and fluorescent substances have been identified in chromatographed extracts of coho skin (Markert and Vanstone 1966).

Chromatographed extracts of chorioids of stingarees contain at least four distinguishable fluorescent entities, one of which (not the main component) is probably isoxanthopterin. Eyes of spur dogs (*Squalus acanthias*) and alligators (*Alligator mississipiensis*) contain a blue fluorescent material, thought to be xanthopterin (Pirie and Simpson 1946), but xanthopterin was not identified in the chorioids of the stingaree in the present study.

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SEASONAL CHANGES IN THE RESPIRATORY METABOLISM OF THE PINFISH (*Lagodon rhomboides*)[1]

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ABSTRACT

Respiratory metabolism of the pinfish was assessed at approximately 10, 15, 20, 25 and 30 C over four seasons. Metabolism-temperature curves from these data indicate that there are considerable differences in the shapes and displacements of the curves; the winter levels are higher than the summer levels. From the same data, at 10–20 C and 20–30 C levels, multiple regressions were calculated to relate the rate of oxygen consumption to weight, temperature and swimming velocity. The respiration-weight partial regression coefficients tend to be quite high (near unity) for the 10–20 C range at all seasons, while the coefficients for the 20–30 C range are of the usual order (0.8). The seeming inconsistency in the respiration-weight coefficients is discussed in terms of an interaction of respiration, weight and temperature that must result in a relatively greater depression of the metabolism of the larger fish at lower temperatures. The respiration-temperature multiple regression coefficients are much higher in the 10–20 C range than in the 20–30 C range, with indications that at the highest temperatures there is little respiratory response to temperature, especially for the largest fish. The respiration-swimming activity coefficients are higher for the lower temperature range. A comparison of laboratory data and direct observations of swimming indicates that this species expends considerable energy over long periods of time at slow to moderate swimming velocities.

From determinations of standard metabolism rates for small groups of pinfish in a large, darkened and enclosed aquarium, there is evidence that the metabolism-temperature relationship

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from 10–30 C is linear and at the same level for a large portion of the year. The standard level is at or near the "Krogh" standard level for many other fishes. The linearity of the standard line on a semilog plot compared to the curvilinear routine metabolism-temperature curves implies that the scope for routine activity declines markedly at higher temperatures and indicates why the smaller pinfish can be more active and survive better at the highest environmental temperatures.

INTRODUCTION

The purpose of this study is the investigation of the nature of seasonal variations in the respiratory metabolism of the pinfish, *Lagodon rhomboides,* with reference to both subtropical environmental temperatures and acclimation temperatures between 10 and 30 C, to body size and to swimming activity.

A coastal and estuarine species like the pinfish is subjected to wide seasonal ranges of thermal and other environmental conditions in its natural range from the U. S. middle Atlantic, Gulf of Mexico and Yucatan coasts. Its natural history is fairly well known (Caldwell 1957), but preliminary studies of growth of southern Texas pinfish as revealed from scale markings showed a pattern that was considerably confusing when compared to Caldwell's data or the usual summer-winter growth patterns of temperate and some subtropical species. Gunter (1950) indicates that some species having similar distributions commonly have slower growing members and smaller individuals along the Gulf coast, in spite of the facts that Gulf temperatures tend to be warmer and the general biological productivity tends to be higher than in east coast waters. This study is thus an attempt to account for any subtropical seasonal changes in metabolism, apart from direct effects of temperature, that would be related to growth and other ecological characteristics.

Although there is a large literature on the nature of metabolism of fishes, there is little information on subtropical species or on seasonal variation, except for winter-summer comparisons. Fry (1957, 1964) and Winberg (1956) contain excellent reviews. Scholander *et al.* (1953) have extensive comparisons between arctic and tropical fishes. There is similarly a large literature on the theory of metabolism and growth as applied to fishes and expressed in mathematical models, which in themselves usually do not consider directly seasonal variations apart from temperature. Ursin (1967) has a detailed review of these models and their applications. That metabolism, measured indirectly by oxygen consumption, can vary between winter and summer and that this variation can have possible adaptive significance for fishes were suggested by Wells (1935). While more recent data have confirmed thermal and latitudinal adaptive characteristics, Wohlschlag and Juliano (1959) and Wohlschlag (1964a) have noted that relationships between oxygen consumption and weight, between oxygen consumption and swimming activity, as well as between oxygen consumption and temperature, can all vary seasonally. By means of simple multiple regression models, these latter studies show how seasonal changes in metabolism accompany normal seasonal ecological changes. Hopefully, these models, with appropriate extensions and modifications, can be useful ultimately in predicting how a species adapts and survives over biogeographic ranges, or over ranges of man-made environmental alterations, or over unusual climatic variations.

PROCEDURE

During 1966–67 pinfish utilized in these studies were from the Aransas Pass at the northern terminus of Mustang Island, Texas (Lat. 27° 50′N., Long. 97° 03′W). Throughout the period the waters were at salinity levels of 32–35 ppt. Winter data for routine and active metabolic measurements were at a temperature range of 13–16 C and are summarized in Wohlschlag and Cameron (1967). Environmental temperature ranges during late April through early May were 20–25.5 C; during late July through August, 29–30 C; and during late October through December, 25–19 C. For these experiments the weights of the individuals ranged from 13 to 111 g. Salinities in both the natural habitat and in the laboratory varied approximately from 32 to 35 ppt.

Freshly caught fish were removed from the Aransas Pass and immediately transferred to a well-aerated, thermally controlled, aquarium to the nearest approximate 5° over a range of 10–30 C. Fish were held at this selected temperature at least 3 days before making either respiration measurements or a 5 C change per 24 hours to another temperature level, which in turn was maintained for 3 days before making either another 5 C temperature change or a set of respiration rate determinations. At any interval, control was maintained to about ± 0.05 C.

Respiratory metabolism was measured in terms of oxygen consumption by individuals enclosed within a circular 10.3-liter clear plastic chamber suspended in the large aquarium by a rotating device. Oxygen levels in the chamber were determined by the Winkler method initially and at three successive 15-minute periods. Initial oxygen concentrations were always near saturation. A simple linear regression through the 4 points over the 45-minute period provided an estimate of the rate of oxygen consumption, from which was subtracted any appreciable rate similarly calculated from a blank run. (A correction of the order of 5 % would be expected in cases when the sea water contained a high level of organic materials when the water was relatively warm.) From 6 to 15 determinations were made at each temperature level during each season.

Temperature was recorded to the nearest 0.01 C. During the 45-minute run the chamber was mechanically rotated at whatever velocity the fish would swim consistently; some fish would remain quiescent, while rarely one would swim at a sustained velocity of nearly 20 m/min. Weights and lengths were also recorded at the end of each run at which time the fish were removed from the chamber.

The data were analyzed by use of multiple regressions relating the log oxygen consumption rates to the log weights, the temperature and the swimming velocity. General descriptions of the conduct of experiments, the analyses of results and critiques are in Wohlschlag (1957), Wohlschlag and Juliano (1959), Wohlschlag (1962a) and Wohlschlag and Cameron (1967).

Because the metabolism-temperature functions were of varying degrees and kinds with respect to curvilinearity and because no relatively simple—and biologically meaningful—function could be found to apply universally to all the seasonal data, the regressions were arbitrarily calculated over the 10–20 C and the 20–30 C ranges within each season.

For the purpose of determining a "standard" respiratory metabolism rate, the insulated aquarium was converted to a respiration chamber by outfitting it with a clear plastic dome that could be clamped and sealed over its top edges. Water, filled to the top of the dome through an inlet funnel, made a total chamber volume of 523.4 l. Water samples for oxygen analyses could be withdrawn while replacement water was drawn in through the funnel to prevent formation of a bubble in the dome. The dome was covered with a flexible insulating blanket of 2.5 cm polyurethane foam between two layers of black polyethylene sheeting. Inside, the aquarium had an inclosed pumping system for circulating the water over temperature and oxygen probes, which in turn activated recorders. An internal aerating system was also utilized so that the dome could remain in place during aeration between oxygen consumption rate determinations or blank runs.

During May and June a series of respiratory measurements was made at 15.5, 21.0, and 26.1 C with small groups of fish. A winter-spring series of data for small groups from mid-January through March was obtained at temperatures of 10.5, 15.6, 19.9, 25.0, 25.0, and 30.4 C. As in the measurements for individual fish, there were three-day acclimation periods at each temperature. Temperatures were controlled to ± 0.1 C. Because of higher respiratory rates, fewer fish were usually required for higher temperatures. The usual protocol was to run the aerators for 2–4 hrs between 4-hr oxygen consumption rate determinations in such a way that all six 4-hour periods could be completed within 2 days at each temperature level. During each of the 48-hour runs the fish were kept continuously under darkened conditions. Rates of oxygen depletion were determined both from the recorder charts and from Winkler analyses made every two hours or oftener used to check oxygen electrode drifts.

The overall rates in mg/hr were originally calculated at ½-or 1-hr intervals to detect circadian rhythms, which were not identifiable. The overall oxygen consumption represents an average of *all* the consecutive hourly rates for six four-hour periods.

RESULTS

In Table 1 are summaries of selected data for each of the four seasons, including winter data of Wohlschlag and Cameron (1967). At each average temperature and routine swimming velocity, the plots of corresponding average log oxygen consumption rates per kilogram are in Figure 1, which illustrates the qualitative nature of seasonal variation in metabolism-temperature relationships.

Multiple regression analyses of these data by 10–20 C and 20–30 C intervals are in the form

$$\hat{Y} = a + b_w X_w + b_t X_t + b_s X_s ,$$

where

$\hat{Y}$ is the expected log mg oxygen consumed per hr,

a is a constant,

b_w is the partial regression coefficient for the increase in $\hat{Y}$ per unit log increase in weight at constant temperature and swimming velocity,

TABLE 1

Pinfish *(Lagodon rhomboides)*. Selected data from seasonal experiments

	No.	Average C	Weight: Smallest (g)	Weight: Largest (g)	Weight: Average log weight	Swimming velocity (m/min): Lowest	Swimming velocity (m/min): Highest	Swimming velocity (m/min): Average	Swimming velocity (lengths/sec): Lowest	Swimming velocity (lengths/sec): Highest	Swimming velocity (lengths/sec): Average	Log oxygen consumption mg/hr: Lowest	Log oxygen consumption mg/hr: Highest	Log oxygen consumption: Average mg/hr/kg
Winter[1]:														
	6	10.06	27	65	1.628	0.37	4.08	1.57	0.05	0.43	0.17	0.779	1.386	2.373
	6	15.16	23	46	1.503	2.60	5.82	3.91	0.34	0.83	0.52	0.861	1.163	2.518
	6	20.31	17	103	1.674	0.00	5.03	2.47	0.00	0.77	0.29	1.033	1.546	2.692
	6	24.98	16	91	1.644	0.00	5.32	3.01	0.00	0.58	0.32	1.072	1.702	2.785
	6	29.65	25	69	1.636	0.00	5.98	3.51	0.00	0.63	0.40	1.310	1.688	2.878
Spring:														
	6	10.73	23	65	1.614	0.00	7.78	4.29	0.00	1.09	0.53	0.507	1.289	2.329
	6	14.67	38	63	1.729	0.00	9.58	6.29	0.00	0.97	0.66	1.110	1.389	2.590
	6	19.83	36	62	1.658	0.00	11.76	7.38	0.00	1.20	0.85	1.308	1.466	2.719
	6	24.86	24	74	1.659	0.00	16.00	6.82	0.00	1.58	0.69	1.197	1.661	2.798
	6	29.78	24	83	1.677	4.09	15.69	9.92	0.38	1.70	1.14	1.285	1.754	2.877
Summer:														
	10	11.87	25	90	1.617	0.00	10.56	2.27	0.00	1.09	0.26	0.193	1.111	2.153
	12	15.81	13	75	1.661	0.00	13.69	6.22	0.00	1.41	0.72	0.215	1.427	2.307
	8	20.12	33	85	1.667	0.00	13.00	7.43	0.00	1.57	0.92	0.976	1.553	2.493
	6	25.62	22	83	1.537	3.77	13.87	8.05	0.54	1.81	1.01	1.007	1.567	2.659
	10	30.07	30	66	1.647	3.27	13.30	7.51	0.33	1.56	0.87	1.094	1.584	2.717
Autumn:														
	6	10.70	14	111	1.906	3.04	14.04	8.44	0.25	1.51	0.84	0.663	1.741	2.393
	12	15.10	33	109	1.978	2.62	13.82	8.88	0.63	1.35	0.79	0.947	1.805	2.434
	14	20.02	43	102	1.873	0.58	14.56	8.97	0.05	1.50	0.87	1.204	1.786	2.625
	14	24.90	34	112	2.034	0.63	15.27	8.65	0.04	1.59	0.76	1.202	1.924	2.637
	7	29.86	31	107	1.906	1.97	14.50	8.91	0.17	1.76	0.87	1.255	1.884	2.769

[1] From Wohlschlag and Cameron (1967).

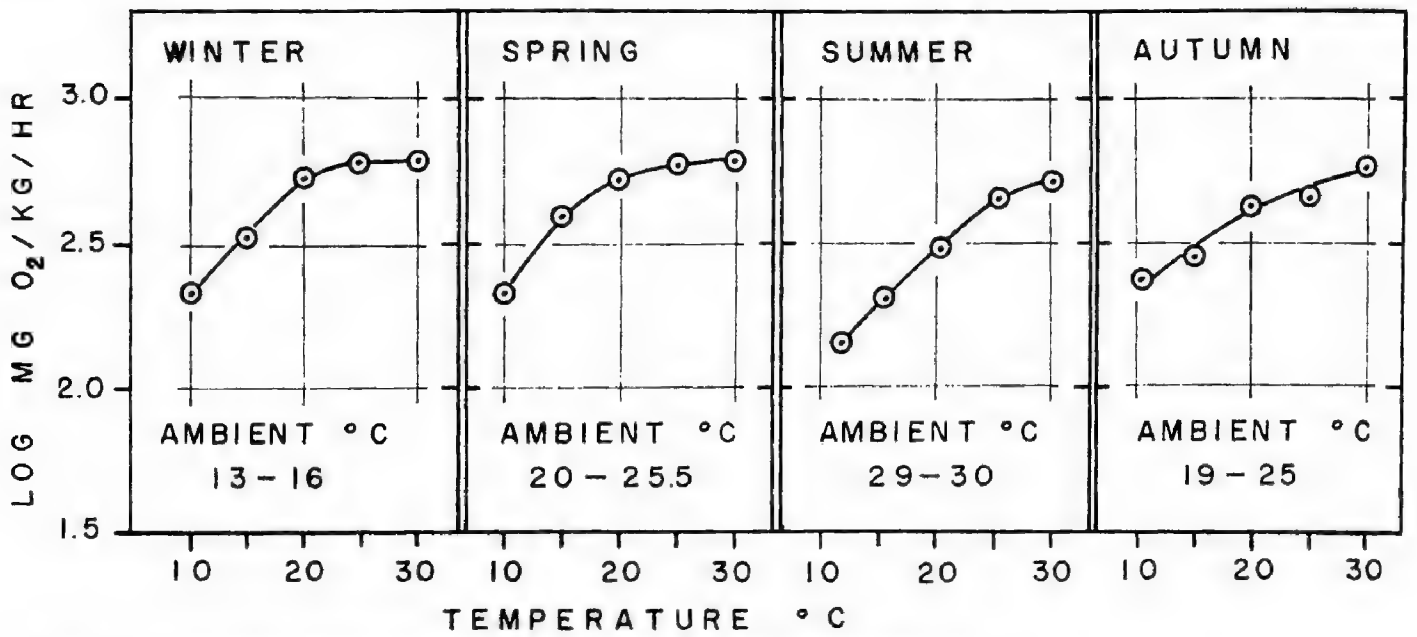

FIG. 1. Routine metabolism patterns of the pinfish for four seasons.

X_w is the log weight in grams,

b_t is the partial regression coefficient for the increase in $\hat{Y}$ for each degree of temperature increase at a constant weight and swimming velocity,

X_t is the temperature in C,

b_s is the partial regression coefficient for the increase in $\hat{Y}$ for each m/min increase in swimming velocity at constant weight and temperature, and

X_s is the swimming velocity in m/min.

The appropriate regressions, including those previously published for winter data (Wohlschlag and Cameron 1967) are:

Winter:

10–20 C; $N = 18$

$$\hat{Y} = -0.8909 + 0.9389\,X_w + 0.0287\,X_t + 0.0302\,X_s \quad (1)$$

20–30 C; N = 18

$$\hat{Y} = -0.3397 + 0.7855\,X_w + 0.0178\,X_t + 0.0108\,X_s \quad (2)$$

Spring:

10–20 C; N = 18

$$\hat{Y} = -1.0006 + 1.0043\,X_w + 0.0389\,X_t + 0.0089\,X_s \quad (3)$$

20–30 C; N = 18

$$\hat{Y} = -0.2931 + 0.7889\,X_w + 0.0162\,X_t + 0.0049\,X_s \quad (4)$$

Summer:

10–20 C; N = 30

$$\hat{Y} = -1.5114 + 1.0321\,X_w + 0.0379\,X_t + 0.0326\,X_s \quad (5)$$

20–30 C; N = 24

$$\hat{Y} = -0.6441 + 0.8183\,X_w + 0.0183\,X_t + 0.0129\,X_s \quad (6)$$

Autumn:

10–20 C; N = 32

$$\hat{Y} = -1.0130 + 0.9715\,X_w + 0.0275\,X_t + 0.0143\,X_s \quad (7)$$

20–30 C; N = 35

$$\hat{Y} = -0.4795 + 0.8381\,X_w + 0.0145\,X_t + 0.0118\,X_s \quad (8)$$

Regression statistics for these equations are in Table 2.

TABLE 2

Pinfish *(Lagodon rhomboides)* regression statistics

Equation	Degrees of freedom	Standard error of estimate	Multiple correlation		Standard errors of estimate for partial regression coefficients					
					Weight		Temperature		Velocity	
			$R_{Y.wts}$	P	b_w	P	b_t	P	b_s	P
Winter:										
10–20 C	14	0.1091	0.9135	0.001	0.1529	0.001	0.0063	0.001	0.0152	0.05<P<0.10
20–30 C	14	0.0514	0.9670	0.001	0.0611	0.001	0.0033	0.001	0.0067	0.1<P<0.2
Spring:										
10–20 C	14	0.1573	0.8372	0.001	0.3393	0.01<P<0.02	0.0107	0.001<P<0.01	0.0118	0.4<P<0.5
20–30 C	14	0.0272	0.9868	0.001	0.0518	0.001	0.0016	0.001	0.0014	0.001<P<0.01
Summer:										
10–20 C	26	0.1201	0.9306	0.001	0.1245	0.001	0.0069	0.001	0.0050	0.001
20–30 C	21	0.1284	0.7742	0.001	0.1772	0.001	0.0061	0.001<P<0.01	0.0080	0.1<P<0.2
Autumn:										
10–20 C	28	0.0904	0.9507	0.001	0.0649	0.001	0.0045	0.001	0.0043	0.001<P<0.01
20–30 C	31	0.1051	0.8749	0.001	0.0612	0.001	0.0049	0.001<P<0.01	0.0048	0.02<P<0.05

TABLE 3

Pinfish *(Lagodon rhomboides)* standard respiratory metabolism data for groups of fish in enclosed 523.4-liter aquarium

Temperature	Number of fish	Weights (g)		Oxygen consumption (mg/hr/kg)
		Total	Average	
Spring-summer:				
15.5 C	14	1205.4	86.1	65
21.0 C	11	479.3	43.6	115
21.0 C	19	921.0	48.5	118
26.1 C	11	720.9	65.5	233
Winter-spring:				
10.5 C	31	1838.9	59.3	42
15.6 C	27	1567.2	58.0	43
19.9 C	29	1717.0	59.2	114
25.0 C	18	994.4	55.2	171
25.0 C	10	664.0	64.4	257
30.4 C	6	355.7	59.3	388

In Table 3 are the results of the oxygen consumption measurements on groups of fish for the determination of standard rates.

Notes on field and laboratory observations of behavioral characteristics, which are of importance in the interpretation of the data, are in the following sections.

DISCUSSION

Techniques

The techniques used in these studies yield results that have been consistent for arctic coregonid whitefish (Wohlschlag 1957), for the temperate centrarchid bluegill (Wohlschlag and Juliano 1959) and for several antarctic nototheniids and a zoarcid (*see* review by Wohlschlag 1964a). The levels of statistical variability for the multiple regressions (Table 2) of this study are also consistent with the earlier studies and of the same order of magnitude. By immersing the respiratory metabolism chamber in the aquarium in which the fish have been acclimated, there is no problem of exciting the fish by transfer to different water or by prolonged handling when they are introduced into the chamber.

The 3-day thermal acclimation periods appear to be more than adequate, judging from a 72-hr trial experiment with the completely enclosed aquarium in which freshly caught, non-acclimated fish dropped to a consistent standard level within 24 hours at a controlled temperature about 9 C below ambient water temperatures. After 3 days above 30 C, the waters undoubtedly tend to become slightly fouled by metabolic products of both the fish and the microorganisms, except in winter; hence 30 C is the upper limit of these experiments. Only in the summer, beginning at 29–30 C, and reducing the temperature by 5 C intervals, does 10 C appear to be below a sublethal level.

Seasonal Differences in Routine Metabolism

The crude data in Figure 1 indicate quite clearly pronounced seasonal changes

in metabolic response to temperature. Both the shape and level of the curves are different from one season to the next. Apart from thermal response to temperature itself, Fry (1964) reviews the rather limited literature on the subject of seasonal influences on respiration of fishes, while emphasizing the importance of including seasonal factors in future work. Data for the antarctic *Trematomus bernacchii*, which lives at a nearly constant temperature of about −1.9 C, indicate that respiration rates change substantially with season (Wohlschlag 1964a). Among other seasonal factors known to influence respiratory levels of fishes are photoperiods (Roberts 1964, 1967), hormonal activities (Hoar 1957, 1959) and feeding activities.

Feeding activities and assimilative processes in relation to respiration and growth might be especially important in the case of the pinfish whose growth rates seem to be irregular with reference to temperature. Examination of scales reveals that annular patterns are confused after about the second year and that there is at least a likelihood that growth rates are depressed during temperature extremes in *both* winter *and* summer. Ursin's (1967) analysis of the Pütter, von Bertalanffy, Beverton-Holt, Paloheimo-Dickie, Ivlev and Winberg mathematical models reveals that respiration rates depend upon the degree of changes in anabolic (growth) and catabolic (maintenance) rates which in turn depend upon diverse—and diversely acting—factors, among which are feeding, assimilation, growth and weight relationships and temperature. If the assumption that seasonal temperature directly or indirectly influences these relationships be acceptable, then it is reasonable that metabolic rates themselves would not necessarily be the same at given temperatures in various seasons. Ursin (1967, Appendix XIII) illustrates diverse means by which temperature could influence growth and, presumably, respiration or metabolism. Because many of these diverse factors, other than temperature, cannot be evaluated easily in a natural setting for fishes, the total outcome of these factors, measured as respiratory metabolism, is a powerful ecological tool for assessing the gross seasonal changes.

The summer and winter levels of respiratory metabolism (Fig. 1) indicate definitely that winter values are higher at all temperatures than the summer levels. The spring levels are nearly the same as those of the winter, but the autumn levels apparently lag in reaching the levels of the spring or winter fish. The pinfish, like the temperate freshwater bluegill (Wohlschlag and Juliano 1959), apparently is cold adapted for winter temperatures. The quantitative levels of respiratory metabolism to which fishes can be cold adapted is considerable as indicated by several studies (*e.g.*, Scholander, *et al.* 1953, Wohlschlag 1964 a, b).

There also can be different thermal responses by fish taken at different times within the same season at similar environmental temperatures. At the 15, 20 and 25 C levels there were two separate autumn collections of 6–8 fish taken about two weeks apart, but at approximately equal temperatures. Although the acclimation regimes were the same, the two groups at each of these temperature levels were rather inconsistent, and the averages likewise do not fit into a smooth transition between 10 and 30 C as do the data for the other seasons. Possibly these two groups of pinfish came from separate populations moving about or through the Aransas Pass at a season when they may have experienced consid-

erable environmental divergencies in terms of temperature, feeding, salinities and possibly spawning influence whose effects might be retained throughout the laboratory acclimation regimes (Breuer 1962).

Because the log oxygen consumption rate-temperature function is nonlinear and seasonally inconsistent, the multiple regression analysis at 10 C intervals is purely arbitrary, though qualitatively informative. To evaluate temperature as a variable in terms of interactions of oxygen consumption rates among weight, activity, and season, there should ideally be curvilinear multiple regression analysis, the analysis of covariance and partial correlation analysis for all the data. Unfortunately no single and biologically simple curvilinear oxygen consumption rate-temperature function is presently known for all seasons. For this reason the regressions calculated over the 10 C ranges are necessarily utilized descriptively.

Multiple Regressions—Weight

While the statistical analyses of the data are straightforward, their ecological interpretations are in some cases less so. The standard errors of estimate for the eight regressions and the multiple correlations (Table 2) are of the usual order of magnitude for experiments of this type, but there are definitely greater variations among the 10–20 C data for the winter and spring fish (Equations 1 and 2) than there are for the 20–30 C data (Equations 2 and 4). For Equations 1 and 3 the standard errors both for the overall equations and for the weight partial regression coefficients, b_w, are considerably higher than they are for Equations 2 and 4.

It is rather remarkable that all the b_w coefficients for the 10–20 C equations are much higher than the b_w for the 20–30 C equations at all seasons. The 20–30 C equations all have b_w values near the 0.8 value found to be about average for a large number of diverse species studied in numerous laboratories (Winberg 1956). The high b_w values could be biased upward by any set of conditions that would either raise the oxygen consumption rates of the larger fish, or lower the rates for the smaller fish, or both. No size-related biases like spontaneous (nonlocomotor) activity (Fry 1967; Basu 1959) or similar behavioral biases were observed at any temperature level to account for the high b_w values at the 10–20 C range throughout the year. Nor do the data in the conventional metabolism-temperature plots of Figure 1 suggest any size-specific biases at the lower temperatures.

Saunders' (1963) data indicate that the respiration-weight coefficient is proportionately higher between 3–10 C than between 10–15 C for starved Atlantic cod, which he studied over a wide weight range. There is thus a distinct possibility that other species of fishes may well have respiration-weight coefficients that change with temperature and size.

Inasmuch as all the b_w values are statistically significant, the comparisons of standard errors of these coefficients (Table 2) are of interest. The standard error of the spring 10–20 C b_w is unusually high. Similarly there is an unusually great variability for the winter and spring bluegill data of Wohlschlag and Juliano (1959 Tables 1 and 2). It is possible that excessive variability of respiration-

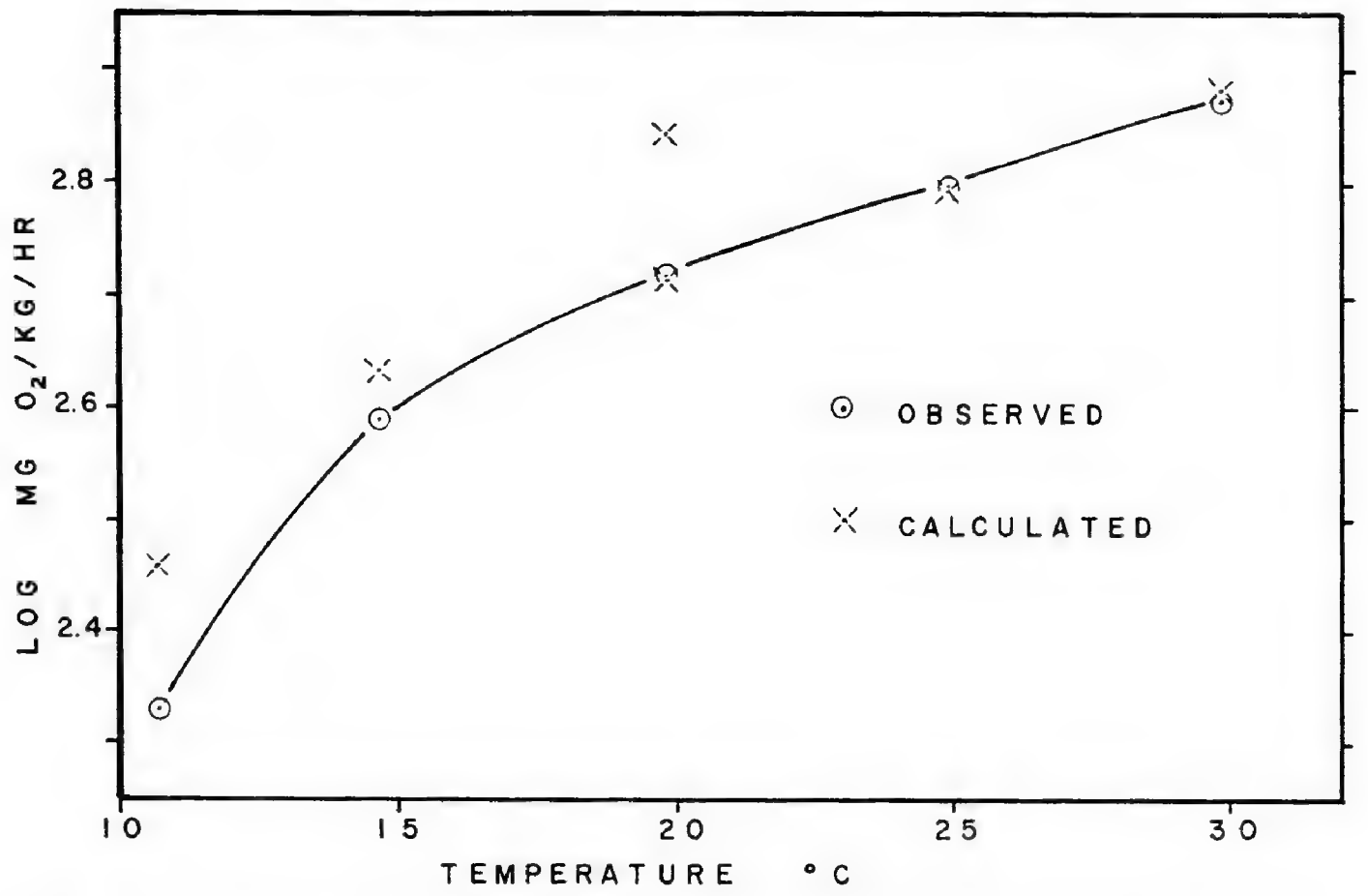

FIG. 2. Comparisons of observed and calculated oxygen consumption rates at different average temperatures for spring pinfish at average weights and swimming velocities. Calculated rates from Equations 3 and 4. (See text).

weight relationships are associated with behavioral changes (*e.g.*, feeding, spawning, migrations) at seasons of low or rising temperatures. Paloheimo and Dickie (1966a) give a summary of influences that may affect b_w values, but there is no incontrovertible evidence on which these influences may be pertinent for the experimental pinfish.

From the various regression equations, it is possible to compare the high b_w values of the 10–20 C equations with the "normal" b_w values of the 20–30 C equations by using the equations to calculate the expected $\hat{Y}$ at each average temperature, weight and swimming velocity for comparison (on a log mg O_2/hr/kg basis) with the corresponding observed values in Table 1 and Figure 1. For the spring data as an example, the two sets of values—observed and calculated—are plotted in Figure 2. The striking effect of the elevated calculated values over the 10–20 C range (from Eq. 3) and the near correspondence of observed and calculated values in the 20–30 C range (from Eq. 4) is obvious from this figure. The comparisons are about the same for the other seasons.

Why the low temperature ranges are so uniformly associated with high b_w values in all seasons has at least one major ecological implication, however. While these regression coefficients may be perfectly representative of the respiration-weight relationships over the small to medium weight range of the experimental pinfish, the calculated $\hat{Y}$ values for the largest locally available pinfish, which would occasionally reach a weight of about 300 g, are much higher (with b_w around unity) than would be realistically plausible either in comparison to the

similar $\hat{Y}$ calculated from the 20–30 C equations or in comparison to the respiratory data in Figure 1 on a kilogram basis. The ecological implications are that such high respiration rates for the largest fish at lower temperatures would have to be "depressed" to be compatible with observations at the higher temperatures, and the statistical implications are that the high respiration rates at the lower temperatures can be pertinent only over the weights used for this series of experiments.

Tentatively, it is likely that the largest pinfish at lower temperature ranges exhibit a respiration-temperature-weight interaction when compared to smaller fish, so that "corrected" b_w values would be linear, but of the usual order of 0.8 instead of 1.0. Although further studies involving both the largest and smallest fishes will be necessary to demonstrate this type of interaction, there are ecological and experimental indications that large fishes at lower temperatures do experience differentially a greater metabolic depression than the smaller fishes, especially under stresses.

In case of winterkills along the Gulf coast, the stress caused by the rapid *rate* of temperature decline apparently incapacitates the larger fish first in the absence of sufficient time for acclimation (Storey and Gudger 1936; Storey 1937; Gunter 1941; Gunter and Hildebrand 1951; Simmons 1962). Stress by very low level pollution at high dissolved oxygen levels has greater effects for metabolic depression on the larger pinfish at the lower temperatures when the b_w is 0.75 compared to 0.94 in unpolluted waters (Wohlschlag and Cameron 1967). There is evidence that larger fishes are the more adversely affected by other stresses like low oxygen levels; *e.g.*, Lowe *et al.* (1967), Cooper and Washburn (1949).

Multiple Regressions—Temperature

The effect of temperature on the respiration rate of pinfish is not unlike its effect on a great many other species (Fry 1957, 1964; Winberg 1956). Few studies, however, show the type of response displacement from season to season indicated by Figure 1. Especially obvious is the downward displacement of the response to lower temperatures during summer, when the fish could not be acclimated within the time limits involved to much less than 12 C. The relatively high winter rates are indicative of cold adaptation (Scholander *et al.* 1953; Wohlschlag and Juliano 1959; Wohlschlag 1964a,b). That oxygen consumption rates are proportionately higher at lower temperature ranges is shown by the comparison of the b_t partial regression coefficients for the respective seasonal 10–20 C and 20–30 C ranges. In each case the b_t are about twice as great for the lower temperature range. All the b_t are statistically highly significant (Table 2), with some tendency toward greater variability for data in the low temperature range. Because of the nature of the experimental equipment and the timing of the acclimation periods, data for temperatures above 30 C are not available, but it is noteworthy that the larger pinfish especially tended to show effects of stress if held at temperatures of about 32 C or greater after a day or so. Probably the respiratory metabolism response to temperature would yield b_t values near zero at about 35 C.

Ecologically, as has been pointed out above, the larger fishes are sensitive to

low temperature stresses; they are likewise sensitive to high temperature stresses. Over shallow grass flat areas in nearby bays, small pinfish can tolerate temporary daytime temperature extremes of nearly 40 C in the summer, but there is no observational evidence that the larger pinfish occupy this habitat at such thermal extremes.

Multiple Regressions—Activity

The effect of swimming velocity on respiration tends to be reasonably linear over at least the small swimming velocity and the temperature ranges indicated in Table 1. Pinfish tend to be relatively slow and erratic swimmers in comparison to active fishes like salmon. Under laboratory conditions, some pinfish of about the average size as used in these studies can swim at a velocity of 20–30 m/min, or less than 3 lengths/sec, for only about 10 min, which has been thus far too short an interval to measure accurately their oxygen consumption rate increases with swimming velocity. In the Aransas Pass the fish were observed during tidal flows or ebbs maintaining their position in relation to pilings at about 20–30 m/min. Under these natural conditions the fish swim in small and irregularly spaced schools beside and behind the pilings. Small schools maintain themselves in the open current, but close observation has revealed that any one fish tends to drop back behind a piling usually in less than 10 min and to be replaced by another moving out from behind the pilings. Behind the pilings the small schools undoubtedly take advantage of the eddies to reduce their locomotor output. The laboratory swimming velocity data are thus representative of a "routine" swimming activity that tends to be maintainable for at least 45 min. Wohlschlag and Cameron (1967) note that the pinfish tends to be aggressive and swim more or less continuously, even at night or under darkened conditions. Thus it is reasonable to suppose that this species has a day-to-day respiration requirement that would be somewhere between a $\hat{Y}$ value calculated for zero swimming velocity and a $\hat{Y}$ value calculated for the average velocities observed in the laboratory.

The b_s partial regression coefficients are of the usual order for fishes of this body shape; *e.g.*, the pumpkinseed (Brett and Sutherland 1965) or the bluegill (Wohlschlag and Juliano 1959). Wohlschlag (1962b) presents b_s values for a variety of fishes and notes that the values tend to be higher at lower temperatures. All the regressions equations for the pinfish in the colder range have higher b_s values than those for the warmer ranges. In terms of hydrodynamical performance, drag force is proportional to density, the skin friction coefficient, surface area, and the square of the velocity. The skin friction coefficient depends variously upon the choice of Reynold's number, which in turn is inversely proportional to the kinematic viscosity. As a consequence of increases in both density and viscosity, the drag force would necessarily be greater at the lower temperatures. A good discussion of these hydrodynamical relationships is in Osborn (1961).

In the rotating chamber the pinfish are generally more sluggish at both the 10 and 30 C levels at which some of them exhibit distress if attempts are made to rotate the chamber faster than their "natural" propensity to swim. Attempts to attain maximum velocities seem most successful between 20 C and 25 C but only for short periods of time. The indications are that the optimal environmental

temperatures are also approximately optimal for maximum swimming velocities. Brett (1956, 1964, 1967) and Brett, *et al.* (1958) review the literature and discuss this and other types of thermal preferenda for several species in relation to the survival values of cruising speeds.

Standard Rates of Metabolism

The various ramifications of the meaning of standard rates have been reviewed by Winberg (1956) and Fry (1957). Saunders (1963) discusses the meaning in terms of fishes in small groups, such as those used for this study under fasting conditions. At all temperatures the values of the rates from Figure 1, even if adjusted to zero swimming velocity, are slightly higher than the standard rates from Table 3. Thus under darkened conditions, small groups tend to be less active metabolically than single fish. The data, as plotted in Figure 3, along with the summer and winter unadjusted routine data, are remarkably linear. The standard lines for both the winter-spring and spring-summer data are, for all practical purposes, strikingly the same. The standard rate is at or below the level

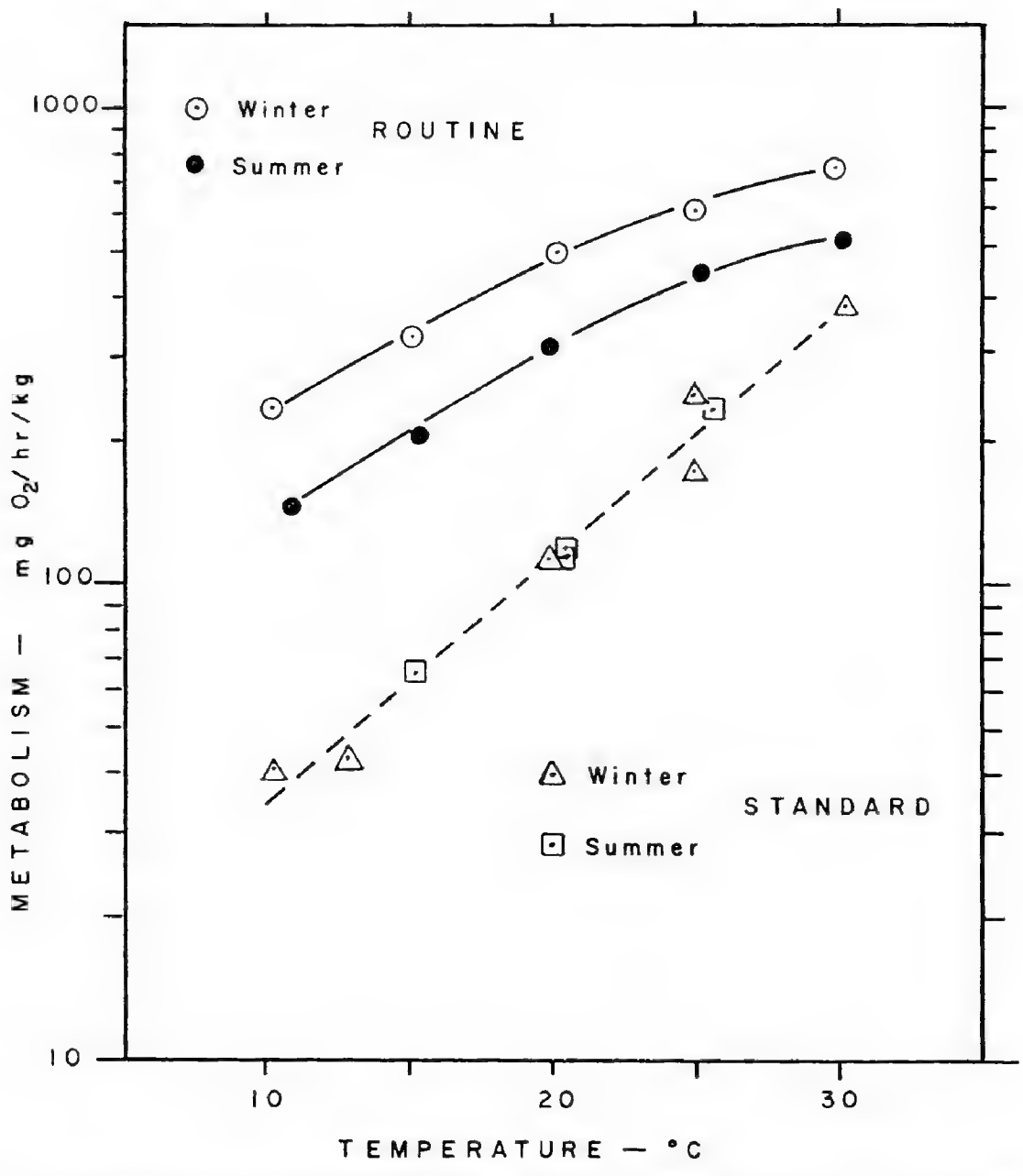

FIG. 3. A comparison of routine and standard metabolism patterns of the pinfish during summer and winter.

of the "Krogh standard rate" (Winberg 1956) between 10 and 25 C, and slightly above this rate from 25 to 30 C.

The differential between the summer or winter routine rates and the standard rate in Figure 3 would be an indication of the "scope for routine activity" as reviewed by Fry (1957). Quite clearly the scope decreases with temperature, which means that excess activity becomes severely limiting, especially for the summer acclimated fish. A discussion of limitations in terms of swimming activity and growth at temperatures above optimum for freshwater sockeye salmon (Brett 1967) is particularly pertinent. The obvious ecological implication is that pinfish during high summer temperatures, and particularly the larger pinfish, would be severely limited in growth and feeding activities especially if low oxygen levels, pollution or other stresses prevailed. This kind of metabolic limitation would explain why annual growth checks on the scales of the pinfish are difficult to identify.

ACKNOWLEDGMENTS

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DISTRIBUTION OF THE EPIBIOTA OF MACROEPIBENTHIC PLANTS

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ABSTRACT

The macrofauna, meiofauna, and epiflora of the southwest Cape Cod eelgrass and algal communities have been quantitatively studied in terms of vertical and peripheral distribution on individual plants, differences in areal distribution in one season, and seasonal changes in distribution patterns.

It was found that faunal abundance on individual plants varies with distance from the bottom and abundance of epiphytes. Some animals vary in peripheral as well as vertical abundance. In a locally varied habitat, amphipod populations show both dispersion and concentration. Areal distribution of the macrofauna varies with kinetic energy and salinity.

Seasonally, populations of mollusks epifaunal on eelgrass show a summer maximum coincident with breeding periods but also have high fall and winter maxima unrelated to breeding. Populations of eelgrass-dwelling amphipods not only exhibit the concentrated winter maxima of mollusks but also have several submaxima throughout the year. The amphipods breed throughout the year; the submaxima are related to the staggered breeding cycles of potentially competing species.

Summer populations tend to be stable and follow Gause's Principle of competitive exclusion. Winter populations tend to be unstable, and competing species follow Gause's Principle less closely. To persist, an epizoal species must be adapted to this physically unstable situation; it must be adapted to both its dispersed summer habitat and to the one winter habitat occupied by all epizoa.

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Widely separated communities on macroepibenthic plants show parallelism in genera and species, much as do infaunal communities.

INTRODUCTION

The epibiota of macroepibenthic plants (macroscopic sea plants living upon and attached to the bottom) has been repeatedly examined sometimes misrepresented, but rarely synthesized. Inadequate taxonomy has hindered American studies. For example, careful compilation of the western Atlantic amphipoda, the dominant epizoal taxon is just now being completed by E. L. Bousfield, National Museum of Canada. European work covers this deficiency, not necessarily for American species, and supplies a wealth of information on mode of life, local distribution, and feeding habits of plant-dwelling epizoans and related forms (*i.e.*, Sars 1895; Enequist 1949; Jones 1948). Unfortunately, the best articles tend to be in the most obscure journals. Many of the best ecological studies have not been concerned with the true epibiota but have dealt with forms dependent on plants only as juveniles. The intensive studies of the American bay scallop (Marshall 1960) represent this type of work. The paper by Parker (1959) exemplifies a different approach; its taxonomic lists of grass flat infauna are especially useful when applied to geologic interpretation. Dahl (1948) is one of the few that considers in detail the distribution and feeding habits of the true epibiota. Many of the plant-dwelling epizoans are fouling organisms as well; summaries such as that of Crisp (1965) give good insight into the biology of this component of the fauna.

Overall, there is a general dearth of knowledge concerning the epibiota of macroepibenthic plants. This study is an attempt by the author to shed some light on the principles and factors governing the occurrence of that biota. These factors are approached by considering different aspects of the distribution of the biota and by attempting to explain distribution patterns through experimentation, comparison with physical parameters of the environment, and observation of mode of life and functional morphology of the dominant members of this biota.

Distribution patterns considered include: distribution on individual plants, areal distribution in one season, seasonal changes in distribution in a local area, and community parallelism. Evidence from these distribution patterns is cited to explain faunal diversity.

In this study, emphasis is placed on the amphipods for a number of reasons. Amphipods are the dominant members of the true epizoa; different species are adapted to the gamut of physical regimes in which macroplants occur. Most adaptations to physical regime are clearly reflected in the exoskeleton, making amphipods ideal for comparative morphology studies. Amphipods exhibited all of the characteristic distribution patterns, from the easily interpreted to the problematical. Finally, amphipods appear to adjust well to experimental laboratory conditions, hence it is possible to support field observations with laboratory experiments.

The bulk of the study was performed during a detailed survey of the benthos of Hadley Harbor, a series of channels and lagoons two miles southwest of Woods Hole, Massachusetts (Fig. 1). Robert H. Parker, Resident Ecologist, Systematics-

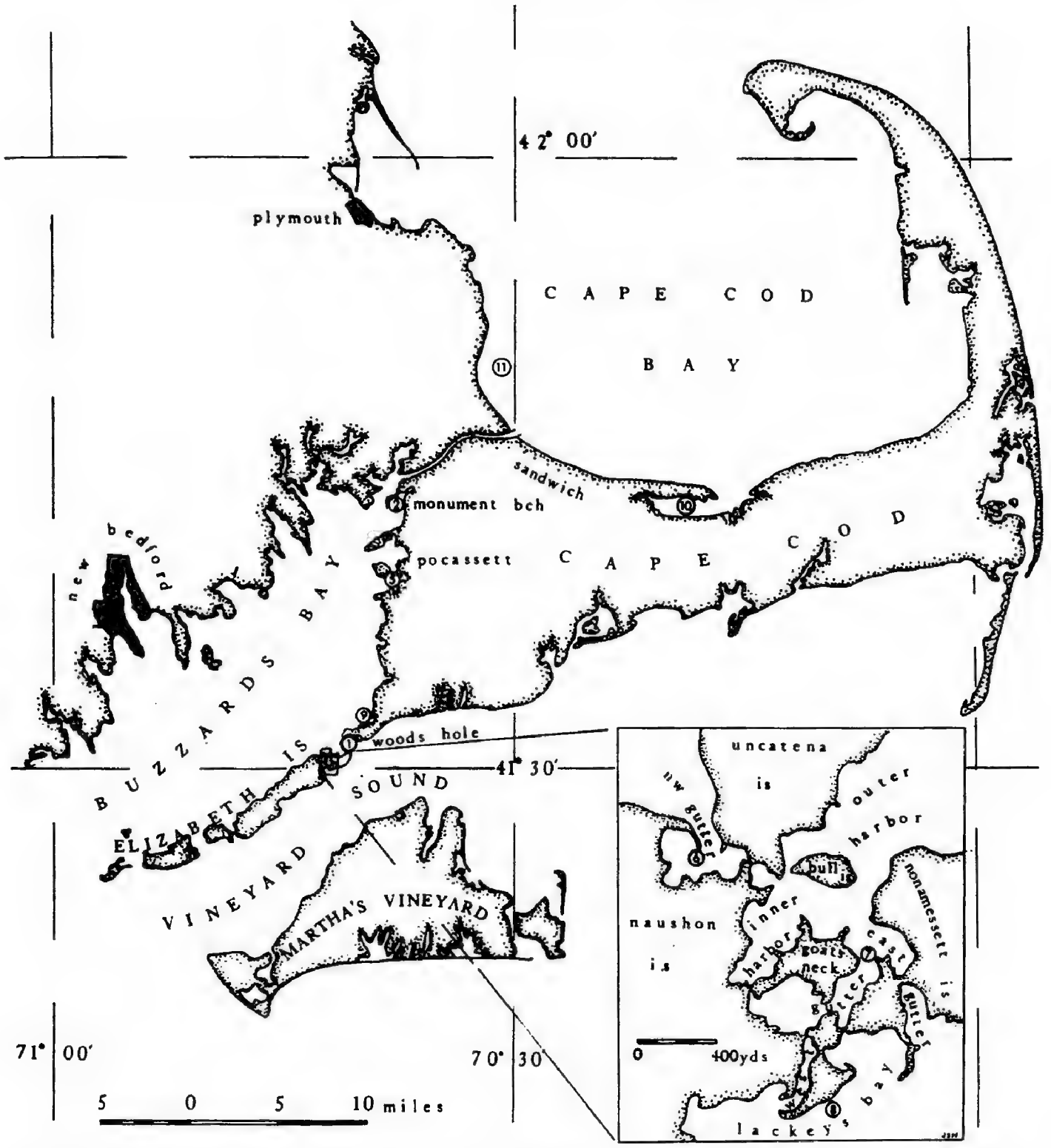

FIG. 1. Index map showing localities sampled. The insert shows the geographic subdivisions of the Hadley Harbor complex that are mentioned in the text.

Ecology Program, Marine Biological Laboratory, Woods Hole, conceived and directed this project.

DISTRIBUTION ON INDIVIDUAL PLANTS

Vertical distribution

It is quite obvious that eelgrass epibiota is not uniformly distributed. In some places, one sees abundant epiphytes and epizoans near the tips of the eelgrass blades; elsewhere, the epibiota is concentrated near the base of the blades, and at still other localities it is uniformly distributed over the whole plant. And in some places, or at some seasons, one finds no epibiota at all. Figures 2 and 3

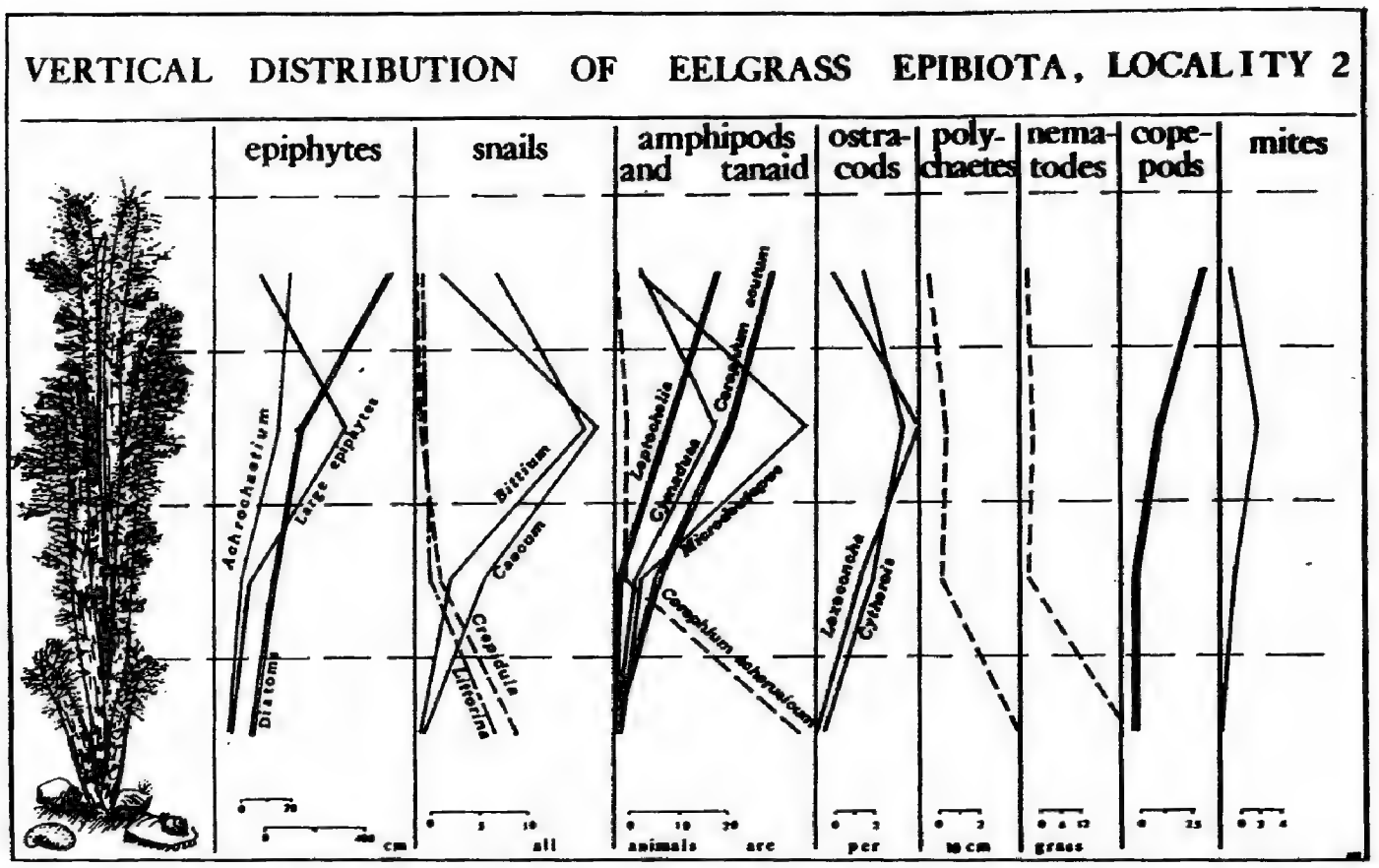

FIG. 2. Vertical distribution of eelgrass epibiota, locality 2, lagoon behind Tobey's Island, near Monument Beach, Mass. Dotted lines indicate animals which generally increase in abundance away from the bottom, dashed lines indicate animals which decrease in abundance away from the bottom, solid lines indicate variation with epiphytes, with single-ruled lines signifying variance with large epiphytes, double-ruled lines signifying variance with diatoms.

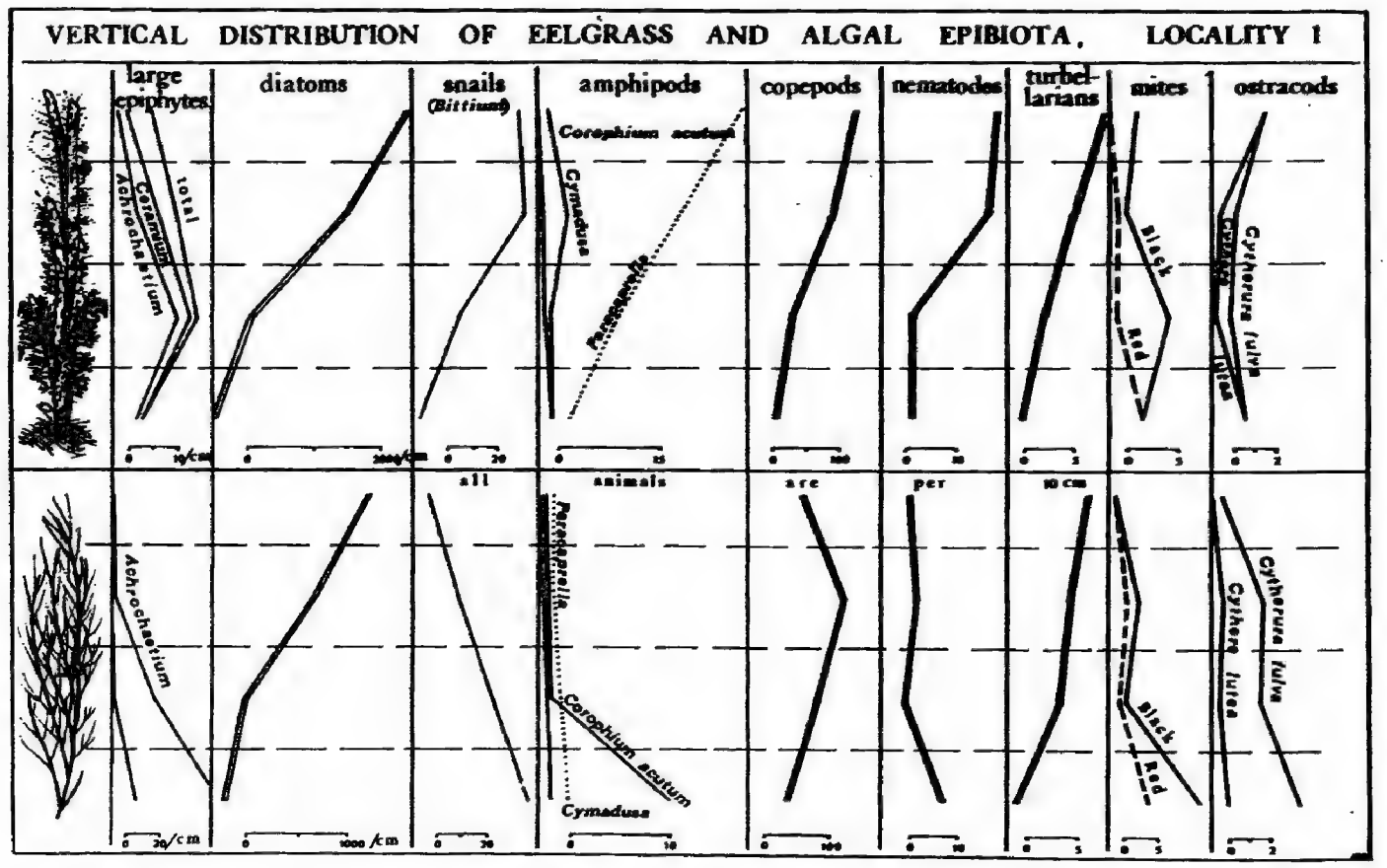

FIG. 3. Vertical distribution of eelgrass and algal epibiota, locality 1, MBL pump dock, Woods Hole, Mass. Ruling of lines is the same as for Fig. 2. Notice that some patterns on the branched algae appear to be anomalous; this is related to the abundance of fine branchlets on the algae because other aspects of distribution are the same as for grass and less-branched algae.

depict the vertical distribution of epibiota on representative macroepibenthic plants.

In these observations, five plants were selected at random from one locality; this size of sample is necessary for statistical identity of replicate samples and is believed to be representative of areas sampled. In the field, plants were covered with plastic bags which were sealed off into four equal portions. The plants were removed from the substratum and immediately transported to the laboratory where all epibiota was scraped from the quartered macroplants. Soft-bodied animals were floated off with a concentrated sugar solution, epiphytes siphoned off, and molluscan residue picked over for living animals; organisms were then fixed in 10 % formalin and transferred to isopropanol the next day. All organisms were classified to the limits of the author's taxonomic knowledge, and individuals per taxon were counted. Number of diatoms and other epiphytes was estimated, and damp weight of epiphytes determined to the nearest tenth of a gram. At first, macroplants were subdivided into 10 cm lengths and biota from each 10 cm segment examined; however, it was found that the same trends could be obtained from quartered plants. Epizoans had less time to abandon their living positions during quartering, hence this procedure was adopted.

Samples forming the basis of this set of observations were taken: (1) off the Marine Biological Laboratory pump dock, Woods Hole, about 9 m from shore, in 1 m of water. The cobble bottom here is strongly affected by waves and tidal currents. Both *Zostera* and a multifilamentous alga (*Desmarestia*?) were sampled. The branched alga *Chondrus crispus* was also examined, but it held no epibiota, and was not sampled.

(2) lagoon behind Tobey's Island, near Monument Beach, Cape Cod, about 16 m offshore, in about 60 cm of water. This area is moderately quiet, being affected only by storms; the bottom is soft and muddy. *Zostera* was sampled here.

(3) in the same lagoon behind Tobey's Island, but in 30 cm of water, about 9 m offshore. *Zostera* again covered the sandy mud bottom and provided samples.

(4) center of an interlagoon channel near Tobey's Island, about 4.5 m from shore, in water 16 cm deep at low tide. The channel is diurnally affected by moderately strong tidal currents and has a gravelly sand bottom. Eelgrass clumps were sampled.

(5) at the edge of a channel near the mouth of the Pocassett Estuary. The *Zostera* sample was taken in about 60 cm of water, 3 m offshore, in a cobble bottom that is diurnally swept by strong currents. Salt water enters the estuary in flood tide but is replaced by brackish water in ebb tide.

(6) Northwest Gutter, Hadley Harbor, in a traverse through a tidal channel 30 cm deep, into a lagoon of the same depth. The channel feels diurnal currents of up to 2 knots, and has a clean, sandy bottom. The lagoon lies behind a spit and is protected from winds by lying in a hollow in a drowned glacial

moraine; water here is quiet and the bottom is very soft and muddy. Eelgrass is abundant in the channel, but sparse and patchy in the lagoon.

(7) East Gutter, Hadley Harbor, near the middle of the channel in 1 to 1.6 m of water. Tidal currents may attain 3 knots in this channel; waves are insignificant. The cobbly sand bottom offered anchorage for *Zostera* and *Sargassum filipendulum*; both were sampled.

(8) Lackey's Bay, Hadley Harbor, about 31 m offshore, in 60 cm of water. Strong waves as well as tidal currents sweep through this area; its clean sands harbored an extremely dense growth of eelgrass and a branched alga, *Spyridia*, both of which were sampled.

(9) Quisset Harbor, Cape Cod, in a nearshore algal bed, in 60 cm of water. The sandy mud bottom here was inhabited by *Fucus* and an undetermined branching brown alga. Collected by W. D. Hope, U. S. National Museum.

(10) Scudder Lane, Barnstable Harbor, in an intertidal sand flat near marsh islands. *Diopatra* (Polychaeta, Onuphidae) tubes studded the laminar sands; dead *Spartina* and epiphytes festooned the tubes; amphipods and spiionid worms inhabited the epiphytes. Epizoan-bearing *Zostera* and *Fucus* were also collected from tide pools.

(11) Cape Cod Bay, 70° 30′ 18″ W, 41° 48′ 6″ N, in gravelly sand, in 21 m of water. The Marine Biological Laboratory supply department dredged numerous sponges *Isodictya deichmannae*; amphipods on the sponges showed similar distribution to those of macroepibenthic plants in shallower waters.

(12) Qualitative samples were taken in tide pools near the Sandwich jetty at the East end of the Cape Cod Canal.

Localities 1, 2, 5, 6, 7, 8, 9, 10, and 11 are indexed in Figure 1; because of the scale of the map, it was not possible to index the other localities.

Three distribution trends are evident; they are emphasized in Figures 2 and 3 by different ruling. Some animals decrease exponentially up the stem (dashed lines), others increase up the stem (dotted lines), but most vary with the epiphytes (solid and double-ruled lines).

Animals which decrease exponentially up the stem include the amphipod *Corophium acherusicum*, red mites, and the snails *Littorina littorea* and *Crepidula fornicata*. Grab samples of the bottom between the grass show these animals to be very abundant in or on the sediment. It is apparent that these grass-dwellers are merely a spill-over from normal benthic populations. Some populations on the branched algae (Fig. 3) decrease up the stem; their abundance, however, is related to the epiphytes which also decrease up the stem.

Others, such as the caprellid amphipods, *Jassa falcata*, another amphipod, and the bryozoan *Bugula turrita*, increase up the stem away from the bottom. Table 1 shows the distribution of *Bugula* on some very long eelgrass from the East Gutter, Hadley Harbor. The four samples, summarized below, were taken early in July, 1965.

Bugula is especially abundant on grass more than 50 cm long, but it is quite rare on short blades. Animals which show this vertical distribution are especially

TABLE 1

Vertical distribution of *Bugula* on eelgrass

Cm. from bottom	0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80
No. of *Bugula*	0	0	1	4	11	12	28	17

abundant near the tips of mature grass blades, but are most abundant on buoys, floats, pilings, and other solid objects placed well up in the water column. All of these animals are suspension feeders; they try to position themselves in as highly agitated water as possible, where strong waves and currents are most likely to bring them food. In dense grass beds, they live high upon long blades, where currents are strongest. Currents are reduced to one-quarter immediately inside the grass bed because the plants baffle the water motion; suspension feeders decrease accordingly.

All the suspension feeders show adaptations to agitated water. Caprellid amphipods have strong third, fourth and fifth pereiopods which grasp tightly around grass blades. Their normal feeding position is erect upon the grass, as depicted in Figure 7. Here, they await food particles with outspread gnathopods. When moving, they anchor their foreparts with their strong gnathopods before releasing and advancing the pereiopods; they inch along as do measuring-worm caterpillars. In so doing, they are not swept away by the strong currents of their preferred habitat. *Jassa falcata* builds detritus tubes, from which it protrudes its coarsely setose antennae. It fishes the water for small organisms upon which it preys, as depicted in Figure 8. Stomachs of *Jassa* yielded unrecognizable crustacean remains and ostracods. *Bugula* colonies are firmly anchored at the base; they resist water movement enough to be important fouling organisms. Fouling organisms prosper with high water exchange (Crisp 1965). Foulers such as *Bugula* do not flourish in the presence of large epiphytes; the epiphytes cut down water exchange. Other fouling organisms such as the ascidian *Molgula*, apparently have similar distribution patterns when they occur on eelgrass. However, they were rare in the areas studied. The foulers are not restricted to macroepibenthic plants, as exemplified by the sample collected at locality 11. Both *Jassa falcata* and *Caprella linearis* were found on the "dead man's finger" sponge, *Isodictya deichmannae; Jassa* built its tubes between the "fingers," *Caprella* inhabited the tips.

The great majority of epizoans show a numerical relationship, significant at the 95% level, to the abundance of epiphytes. In Figures 2 and 3, their vertical distribution is depicted by continuous lines. The epizoans that vary as do large epiphytes show solid lines; those varying with diatoms show double-ruled lines. The presence of the epizoans is obviously related to the presence of the epiphytes. Samples from Lackey's Bay, Hadley Harbor (locality 8) clearly illustrate the close association of epiphytes and epizoans and the independence of both to the macroscopic plants which form the substratum.

In these samples, the macroscopic plants *Zostera* and *Spyridia* were divided into 10 cm lengths, and the number of *Microdeutopus* (an amphipod) tubes per

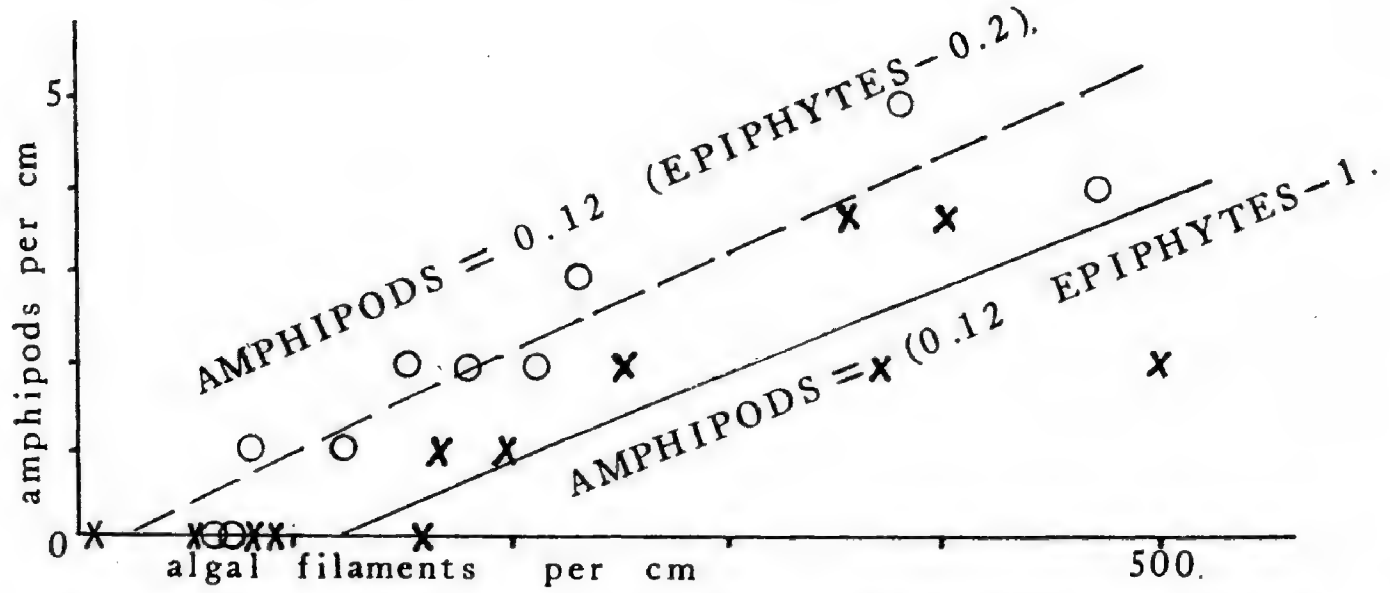

FIG. 4. Numerical relationship of amphipods to epiphytes at locality 8 (Lackey's Bay). X's indicate samples from eelgrass, O's indicate samples from *Spyridia*, a highly branched alga.

cm and large epiphytes per cm were counted. The abundance of amphipods correlates significantly with the abundance of epiphytes.

The amphipod-epiphyte relationship is depicted in Figure 4; notice that the slopes of both curves are the same. This indicates that the number of amphipods is directly related to the abundance of epiphytes, independent of substratum. There are more amphipods initially on *Spyridia* than *Zostera* (slope intercept falls nearer the origin) because the alga is highly branched and initially provides a more favorable habitat for the amphipods. Beyond this initial factor, however, the relationship is the same. Both macroscopic plants happened to be convenient substrata for the same species of epiphytes and associated amphipods.

Three hypotheses were proposed to account for the epizoan-epiphyte relationship: (a) there is a nutritional relationship, (b) the epiphytes conceal the epizoans from predators, (c) the epiphytes trap detritus and provide a framework for the epizoans' dwelling structures. The hypotheses were then tested in the laboratory, as described below.

At first, it was believed that the animals were grazing upon the epiphytes. Observation of feeding habits and examination of stomach contents bore this out for the relatively rare amphipods *Ampithoe longimana* and *Dexamine thea*, and the tanaid *Leptochelia savignyi*, all of which consume great quantities of diatoms. These animals eat their way into diatom clumps, where they reside; globules of yellowish or greenish diatom oil are commonly seen in their bodies. However, the commonest animals, including all the abundant amphipods, ostracods, copepods, and snails, have a more interesting interrelationship with the epiphytes. Observation of their feeding habits shows that they use the setae of the second antennae to comb detritus from the epiphytes; they then remove the detritus from their antennae with the mouthparts. The amphipods pick over the detritus, selecting the particles they choose to eat; the remainder is bound into their characteristic dwelling tubes. Amphipods also pick up large plant fragments with the inner margins of the gnathopods (large, anterior claw-like appendages), scraping off the detritus with the mouthparts. *Microdeutopus* holds the long particles parallel

to the body, whereas *Corophium* holds the same sort of particle transverse to the body with the large second antennae. *Corophium* supplements its diet by suspension feeding. Members of this genus have long setae on the gnathopods; these strain food particles from pleopod-generated currents entering the anterior portion of the tube. In this way, *Corophium* can feed without leaving the tube, an important adaptation to its mode of life, as will be discussed shortly. Examination of the stomachs of about 10 specimens each of *Microdeutopus damnoniensis*, *M. gryllotalpa*, *Cymadusa compta*, *Corophium insidiosum*, and unidentified harpacticoid copepods supported the feeding observations; all stomachs contained 0.1 to 0.3 mm diameter detritus particles and broken plant fibers. *Corophium acutum* also contained a few diatoms. The snails *Bittium* and *Lacuna* were also observed to be detritus feeders.

A starvation experiment indicates that the above crustaceans are only preferential detritus feeders; when their preferred food supply runs out, they become omnivorous. If these crustaceans are supplied only with clean epiphytes, from which the detritus has been removed, they consume the epiphytes after 5 ± 2 hours. After 14 ± 5 hours, they resort to cannibalism. Such starvation is unusual in their natural habitat which abounds in detritus. Accordingly, most of these animals are preferential selective deposit feeders, cleaning detritus from the epiphytes amidst which they dwell.

The fact that amphipods do not normally eat epiphytes raises the question, "Do the epiphytes benefit from this association?" The following experiment gave positive results. Three identical finger bowls of seawater were placed under a 100 watt lamp which was turned on at 8 A.M. and off at 10 P.M. each day. An epiphyte-laden stalk of eelgrass was placed in each bowl. Two stalks were covered with detritus scraped from plants taken from the source bed of the experimental stalks; one stalk was washed clean of detritus. Amphipods were introduced into one dish with the detritus-laden epiphytes; the other held no animals. After 3 days, the detritus-laden epiphytes in the bowl with no amphipods began to turn brown and break apart; the cleaned epiphytes in the other dishes remained healthy and green. It is concluded that the animals are required to keep the epiphytes clean and healthy. The experiment was successfully replicated two times.

Detritus-feeding habits of the amphipods have been known for some time; they were carefully worked out for soft-bottom inhabitants by Dahl (1948) and Enequist (1949). The amphipod-epiphyte interrelationship is one of mutualism on the protocooperation level; both members benefit when interacting, but interaction is not obligatory. As discerned by Dahl and Enequist, amphipods can glean detritus from other sources, and epiphytes can be cleaned by physical agitation of the water. Both interaction and non-interaction situations are apparent in nature.

Casual observation of epizoan color patterns leads one to suspect that the epiphytes provide some concealment for the free-living epizoans, nearly all of which are protectively colored olive-grey with dark vertical bars. The color patterns of the common amphipods *Microdeutopus* or *Corophium* are representative of this type of protective coloration. Experimentation supported the suspicion that epiphytes conceal epizoans.

TABLE 2

Results of *Fundulus* predation experiment

	Bowl #1 no plants	Bowl #2 *Zostera*, no epiphytes	Bowl #3 *Zostera*, 500 epiphytes per cm
Trial 1	6	23	1 amphipods eaten
Trial 2	5	21	6 after 1 hour
Trial 3	0	14	4

In this experiment, replicated three times, killifish (*Fundulus*), major predators on amphipods, were starved for four days before being placed in each of three finger bowls. *Microdeutopus*, a protectively-colored amphipod, was the experimental prey. One test bowl held 25 *Microdeutopus* and no plants, another held 25 of the amphipods on an epiphyte-free stalk of eelgrass, the third held 25 animals on a densely epiphytized eelgrass stalk. One hour after the fish were introduced, the uneaten amphipods were counted. Results are tabulated in Table 2.

In the bowl with eelgrass and no epiphytes, a total of 58 of 75 amphipods were picked off the grass by the fish, whereas in the bowl with the eelgrass holding about 500 epiphytes per cm, only 11 amphipods were eaten. The rest successfully hid amidst the epiphytes. In the bowl with no plants, only 11 of 75 amphipods were eaten because they settled to the bottom, where they were not seen by the top minnows. The few individuals which swam about were consumed.

Observations of living habits reveal that many amphipods use the uneaten detritus for building dwelling tubes. There is a direct numerical relationship between epiphyte abundance and detritus retained by a grass blade. Detritus counts, averaged per epiphyte, reveal that each 5 mm epiphyte strand traps 1.6 particles of detritus 0.1 to 0.5 mm in diameter. And the more detritus, the greater percentage of amphipods building tubes. An experiment relating abundance of detritus to percentage of amphipods building tubes revealed an interesting aspect of their domicolous behavior. Varying amounts of detritus were evenly spread over square-centimeter ruled petri dishes; 20 amphipods were then introduced, *Corophium* in some experiments, *Microdeutopus* in others, and percentage of amphipods building tubes was then determined. The results, depicted in Figure 5, indicate that more amphipods build tubes when more detritus is available (and more detritus is available, of course, when there are more epiphytes). However, not all the amphipods constructed tubes, even when there was a superabundance of detritus. The reason is probably not territorial spacing because tubes are randomly, not evenly, distributed, both in the experimental dishes (Fig. 5) and on plants in the field.

Substratum choice experiments demonstrated that amphipods of the species *Microdeutopus gryllotalpa*, *M. damnoniensis*, *Corophium acutum*, and *C. insidiosum* all prefer epiphyte-bearing macroplants to other substrata, including organic detritus, their preferred food. In the light, they apparently locate their preferred substrata by sight. When placed in the center of a 15 cm finger bowl containing fragments of epiphyte-bearing eelgrass and algae, non-epiphytic eelgrass and algae, and a pile of detritus, 22 of 26 amphipods chose the epiphytic macroplants;

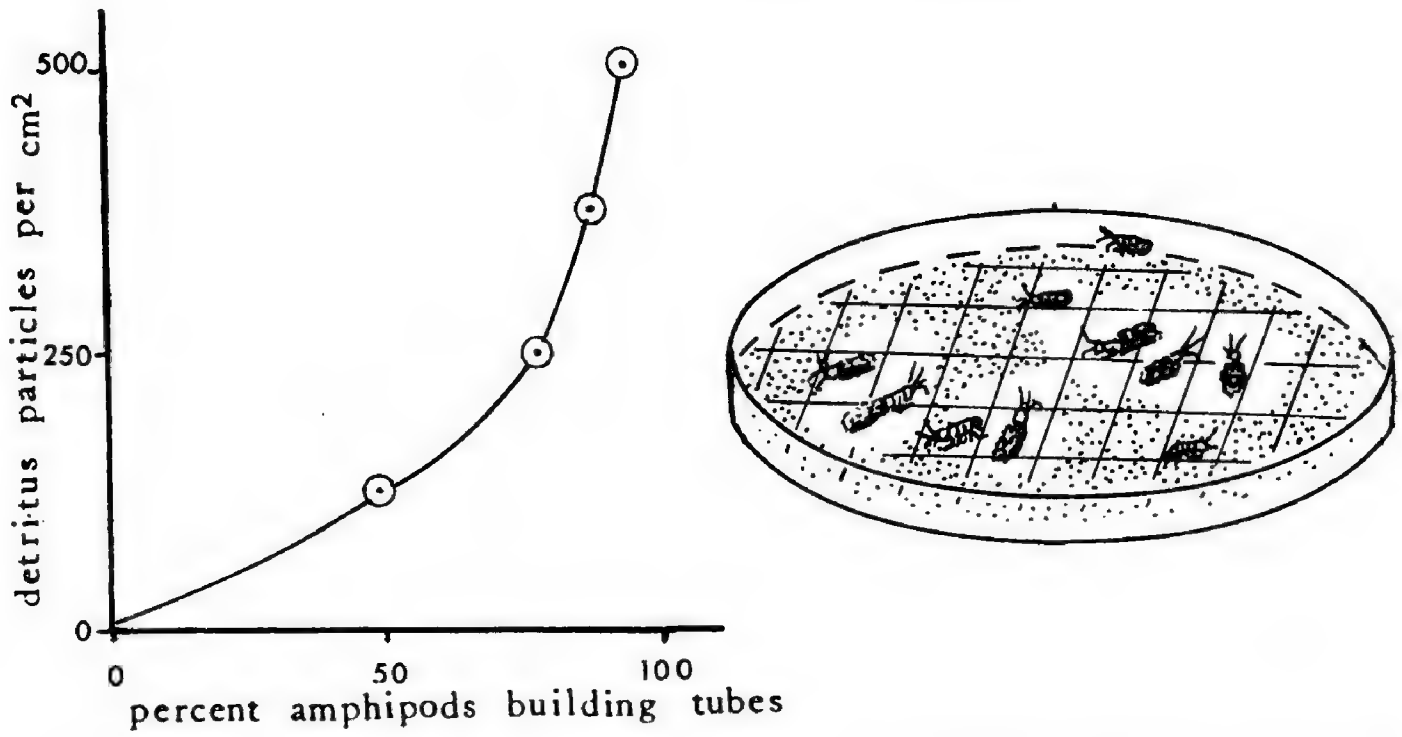

FIG. 5. Relationship of tube construction in amphipods to abundance of detritus. Even with a superabundance of detritus, not all amphipods build tubes.

all rejected the detritus. The amphipods first swam in random circles, mostly clockwise, until they were about 2 cm from an epiphytic macroplant, at which time they headed straight for the target. During their random explorations, which took an average of 3 minutes, they passed-by plants with no epiphytes, and the detritus. Thirteen of twenty amphipods chose epiphyte-bearing plants when all the experimental substrata were tightly sealed in plastic sacks; under these conditions they could not use chemoreception. With experimental plant substrata exposed, but in a darkroom, 40 of 60 amphipods chose epiphyte-bearing algae and eelgrass over plain substrata; however, they took between 1 and 2 hours to make their choice. Touch, not distant chemoreception, seems to be important in making this choice; all of the experimental animals ignored water containing juices of crushed epiphytes.

Abundance of epibiota varies with age of the eelgrass leaves. The Marine Biological Laboratory marine ecology class collected 57 eelgrass plants in June, 1965 from the current-swept and quiet portions of the Northwest Gutter, Hadley Harbor (locality 6). They found that epiphytes were abundant on 470 outer eelgrass leaves that had overwintered, but were abundant on only 4 newly grown inner leaves. Two hundred fifty-nine spring-growth leaves, about two months old, held abundant epiphytes. Conversely, 582 of the newly-grown inner leaves totally lacked epiphytes, whereas only 375 older outside leaves lacked epiphytes. Distribution of *Microdeutopus* and *Bittium* closely followed the epiphyte pattern; there were 88 amphipods and 14 snails on outside leaves, 41 amphipods and 6 snails on moderately epiphytic inner leaves, and 3 amphipods and no snails on newly-grown center leaves. Both genera of animals are freely motile, and could easily move to the center leaves, but they remained on the strongly epiphytic outer leaves. Such a situation is illustrated in Figures 2 and 3. Undoubtedly, as inner leaves become colonized by epiphytes, they are colonized by epizoans as well. The exact epi-

biotic succession on *Zostera* has not been determined but probably does not parallel the *Sargassum* fouling succession of bacteria-animals-algae reported by Conover and Sieburth (1964, p. 150) because most animals do not move onto *Zostera* blades until the fouling algae are long enough to trap detritus.

Abundance of the plant dwelling snails *Bittium laternatum* and *Lacuna pallidula*, as well as that of climbing ostracods *Hirschmannia viridis*, *"Cythere lutea," Cytherura fulva*, *Cythrois zostericola*, *C. pusilla*, *Loxoconcha impressa*, and *L. guttata*, fluctuates with abundance of larger epiphytes. *Ceramium*, *Achrochaetium*, *Polysiphonia*, and *Champia* are the commonest of these. Behavior of the ostracods was not studied extensively, but they appear to be detritus cleaners, as are the snails. Abundance of smaller epizoa, including copepods, nematodes, and turbellarians varies with the smaller epiphytes, principally diatoms (double-ruled lines, Figs. 2 and 3). Copepods were hard to observe under the microscope, as most were photonegative and spent a good deal of their time trying to escape the light. Their stomach contents were mostly detritus; apparently they clean detritus from the diatoms as the amphipods do for the larger plants. Turbellarians are only indirectly related to the epiphytes; Dr. Louise Bush (personal communication, 1965) reports that they prey upon copepods. Hence, their abundance reflects that of their prey and is only incidentally related to epiphyte abundance. Epibiotic nematodes are always found in clumps of detritus; their abundance varies with epiphytes because of the detritus-trapping properties of the tiny plants.

Peripheral distribution

A peripheral as well as vertical zonation occurs when macroplants are colonized by long epiphytes such as *Ceramium*. Some of these epiphytes may be several centimeters long and may themselves be colonized by *Synedra*, a straight-chain diatom. When long epiphytes are present, a common occurrence, the amphipods *Microdeutopus* and *Cymadusa* live closest to the stalk of the macroplant, and the snails *Bittium* and *Lacuna*, as well as amphipods of the genus *Corophium*, live near the periphery. In this way, the feeding territories of these detritus consumers are separated in space. The amphipods aggressively maintain this zonation by pinching intruders of other species with their second antennae, driving them out of their domains. The snails can successfully inhabit the peripheral regions of the epiphytes because they are relatively immune to attacks by fish. A feeding experiment with starved *Fundulus* and *Apeltes* (stickleback) revealed that the fish quantitatively take amphipods before attacking snails. In the wilds, the soft-bodied amphipods are protected from fish predation by living near the stalk of the macroplant. *Corophium* is able to exist on the periphery because it obtains some of its detritus by suspension feeding within the tube; its setose gnathopods make an effective basket for trapping detrital particles entering the anterior portion of the tube (Hart 1930). When detritus is scarce, *Corophium acutum* supplements its diet with diatoms, as indicated by examination of stomach contents.

Nematodes are commonly concentrated near the macroplant stem; their occurrence is related to the greater quantities of detritus which tend to accumulate near the stem. On the other hand, mites, harpacticoid copepods, climbing and swim-

ming ostracods were observed to move freely through all portions of the epiphytes, showing no preference for peripheral or central regions.

AREAL VARIATION

Many members of the "grass bed" fauna are not true members of the epibiota, but, as mytilids and pectens, are bottom dwellers, too large to reside upon the plants except at early stages in their life history. This study considers only the areal distribution of the smaller macrofauna which spend most of their lives on plants—true epibiota. Table 3 summarizes the feeding habits and mode of life of species considered in detail in the succeeding discussion.

Details of distribution of this fauna were studied over the years 1963 to 1965 in the Hadley Harbor area; this area is indexed in Figure 1. In Figure 6, notice the summary of environmental parameters; these are important in understanding the distribution of the epibiota.

Strong waves sweep into Lackey's Bay, open to Vineyard Sound on the southeast; waves from Buzzards Bay beat upon the northeast and northwest shores of Uncatena Island (Fig. 6-A). All other shores are protected from strong waves, but the channels (East, West, and Northwest Gutters) feel the effect of diurnal tides in currents which attain velocities of up to 5 knots. Here, gravel and boulders afford attachment for an ever-changing algal flora. Sands and muddy sands in the wave-swept open coasts and in the gutters afford rooting-places for abundant *Zostera* (Fig. 6-D). In the muddy lagoons, the water is everywhere less than 1 m deep. There is enough light for grass to flourish, but water exchange is so slight that the grass, which takes its nourishment from the water as well as from the

TABLE 3

Mode of life of feeding-preference groups

Species	Motility	Peripheral distribution
Suspension feeders (increase up the stem)		
Bugula turrita	sessile	central, abhors long epiphytes
Jassa falcata	domicolous	central to peripheral, abhors epiphytes
Caprella geometrica	highly vagile	peripheral
Paracaprella pusilla	highly vagile	peripheral
Grazers on epiphytes (relatively scarce, vary with epiphytes)		
Dexamine thea	highly vagile	central
Leptochelia savignyi	highly vagile	central
Ampithoe longimana	highly vagile	central
Detritus-cleaners (abundant, vary with epiphytes)		
Microdeutopus gryllotalpa	domicolous, vagile	central
M. damnoniensis	domicolous, vagile	central
Corophium acutum	mostly domicolous	peripheral
C. insidiosum	mostly domicolous	peripheral
Cymadusa compta	vagile	central
Bittium alternatum	vagile	peripheral
Lacuna pallidula	vagile	peripheral

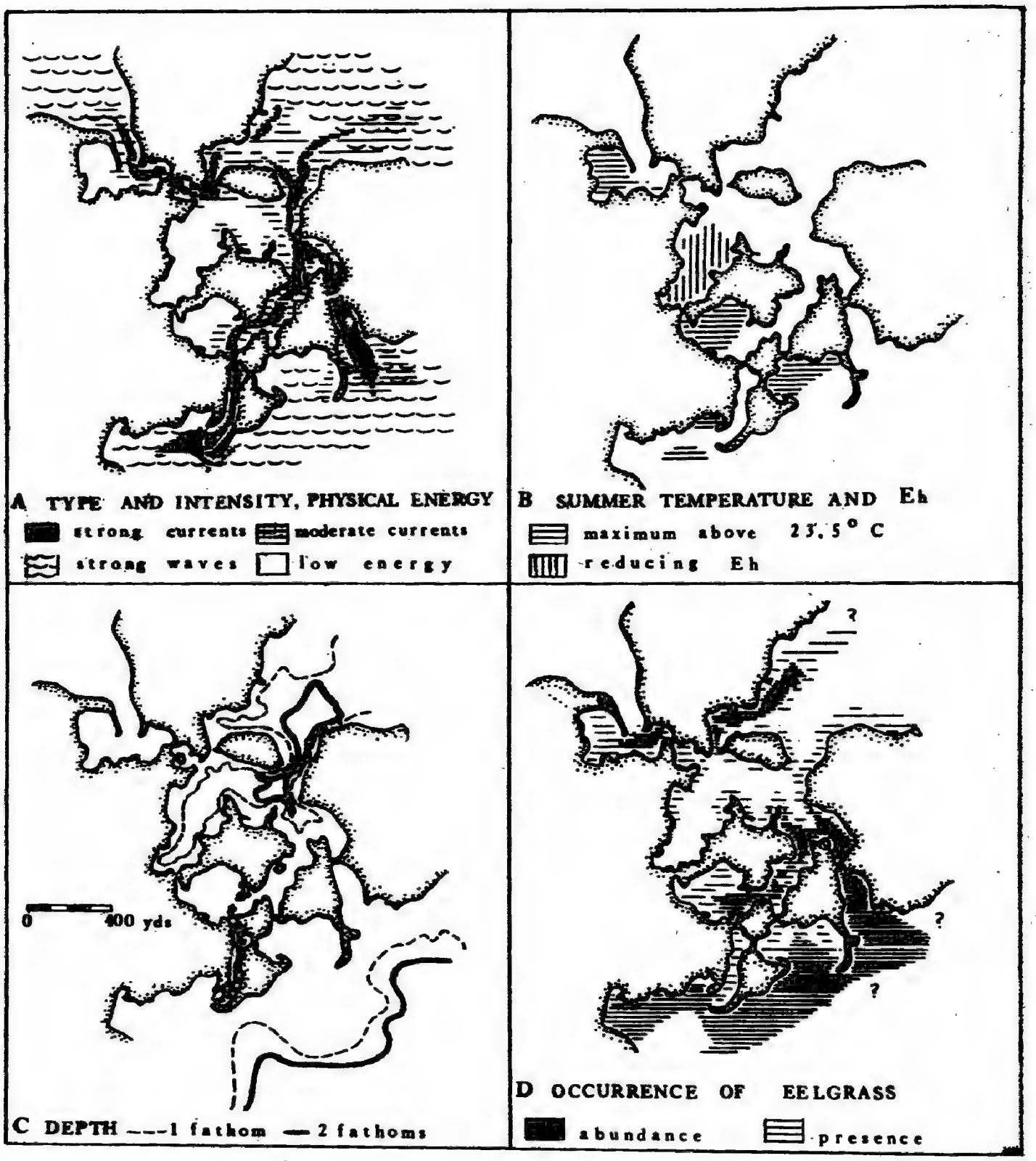

FIG. 6. Physical and biological parameters of the Hadley Harbor complex. A. Type and intensity of physical energy. Strong currents follow the main channels; low energy areas have weak waves that feel bottom in about a foot of water or less and currents too gentle to record on a current meter. B. Summer temperature and Eh. All areas of high summer temperatures are shallow lagoons except the sandflat at the southern entrance to West Gutter. The enclosed Inner Harbor developed reducing conditions in the water column during the summers of 1964 and 1965. C. Depth. The small pockets of deep water in the west and northwest gutters are kettle holes in this drowned moraine. D. Distribution of eelgrass. Areas of abundance have dense grass cover throughout the year; areas of presence have sparse or patchy grass cover in summer, only. The unvegetated area at the southern entrance to West Gutter is a sandflat, exposed at low spring tide. Lack of vegetation in other areas is explained in the text.

soil, can do no more than form a sparse ground cover. Summer water temperatures in these shallows may be a critical limiting factor to some of the epibiota; in sunny days during July and August, temperatures are commonly as high as 24 C (Fig. 6-C), and Allee (1923) has reported temperatures of 32 C. In addition to being quiet, the Inner Harbor is up to 3.6 m deep (Fig. 6-C) and develops reducing conditions in the water column (Fig. 6-B) which inhibit epibiota. Macroepibenthic plants can establish themselves only in the shallows of this area. Its soft bottom holds a deposit-feeding infauna; the sparse epibiota of the center is restricted to floats, pilings, beer cans, and other human-introduced objects.

Hadley Harbor project sampling and preparation procedures will be discussed in detail in a forthcoming paper by R. H. Parker; methods are only outlined here. As many as 18 physical parameters (dependent on the usual perversity of electronic sampling gear) were measured weekly at 10 to 15 physical stations, chosen to provide coverage of the entire habitat. Measurements relevant to the epifauna are summarized in Figure 6. Four biological samples were taken each week with a 1/25 M^2 Van Veen grab sampler. In the laboratory, grab samples were washed through 4, 2, 1, 0.5 and 0.25 mm mesh screens. Animals were mechanically separated with concentrated sugar solution or picked out; they were then fixed for 24 hours in 10% formalin before being transferred to isopropanol. Animals were then identified to lowest taxon possible, sized, and sexed. Data were reduced manually; this provided more insight into areal and seasonal changes than other means of data reduction.

Discussion of animal distribution within one season will be reserved to the summer of 1964. Conditions in summer are the most stable, and the fauna is not adjusting to season fluctuations. First, summer distribution of the common suspension feeding amphipods *Jassa*, *Caprella*, and *Paracaprella* is considered. *Caprella*, a large robust form, occurs in the outer, wave-swept areas of Lackey's Bay and Uncatena Island; whereas *Paracaprella*, a much smaller form, lives in the gutters, in areas affected only by strong diurnal currents (Fig. 7). The range of *Jassa* (Fig. 8) overlaps that of the caprellids, but it reaches its maximum abundance in areas not inhabited by the caprellids. It is domicolous, not actively vagile as the caprellids, but shows adaptations which enable it to inhabit quieter waters and still survive by suspension feeding. Its antennae are evenly covered with long setae; when spread in normal feeding position (Fig. 8) they make an effective net. This mode of feeding allows *Jassa* to cover more surface area than the caprellids, compensating for the lower probability of detrital particles moving past a given point in the quieter waters it inhabits.

The next aspect to consider is the distribution of the five commonest detritus-feeding amphipods: *Microdeutopus damnoniensis*, *M. gryllotalpa*, *Corophium acutum*, *C. insidiosum*, and *Cymadusa compta* (Figs. 9, 10, 11, 12 and 13). Their abundance does not necessarily coincide with greatest abundance of macroepibenthic plants (Fig. 6-D), although these species are virtually restricted to such areas. An important thing to notice is that their populations show areas of presence (horizontal lines only) and abundance (cross-ruled lines), but there are few samples with intermediate numbers of individuals. In areas of presence, the re-

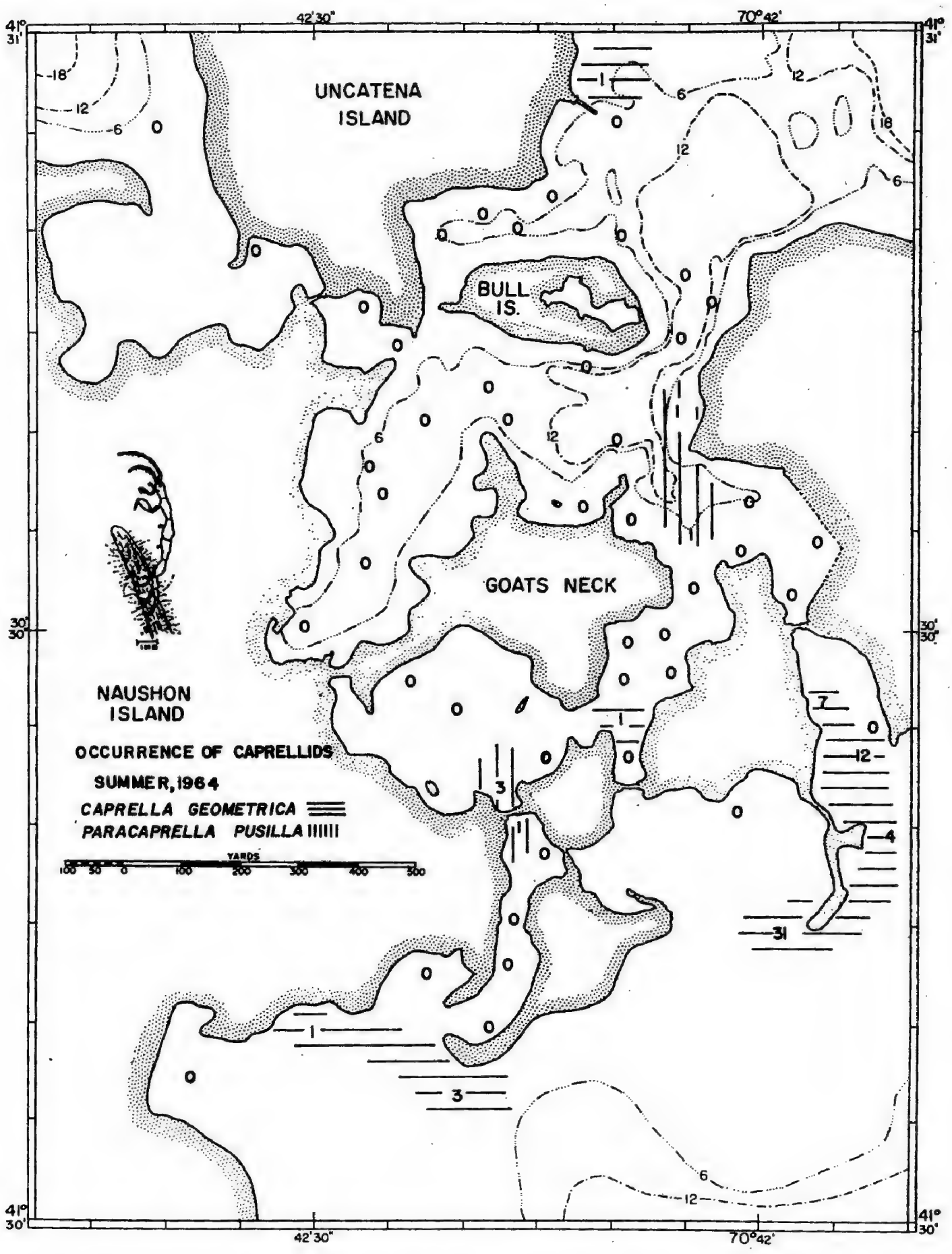

FIG. 7. Occurrence of caprellid amphipods, summer 1964. Their ecological niche is similar to that of *Jassa falcata* (Fig. 8); spatial ranges of these forms show little overlap. The insert shows the normal mode of life for these animals.

lationship of individuals per station and to number of stations, follows a Poisson distribution; that is, there are many stations with few individuals, and less with a fair number of individuals (Fig. 14). This normal distribution breaks down in

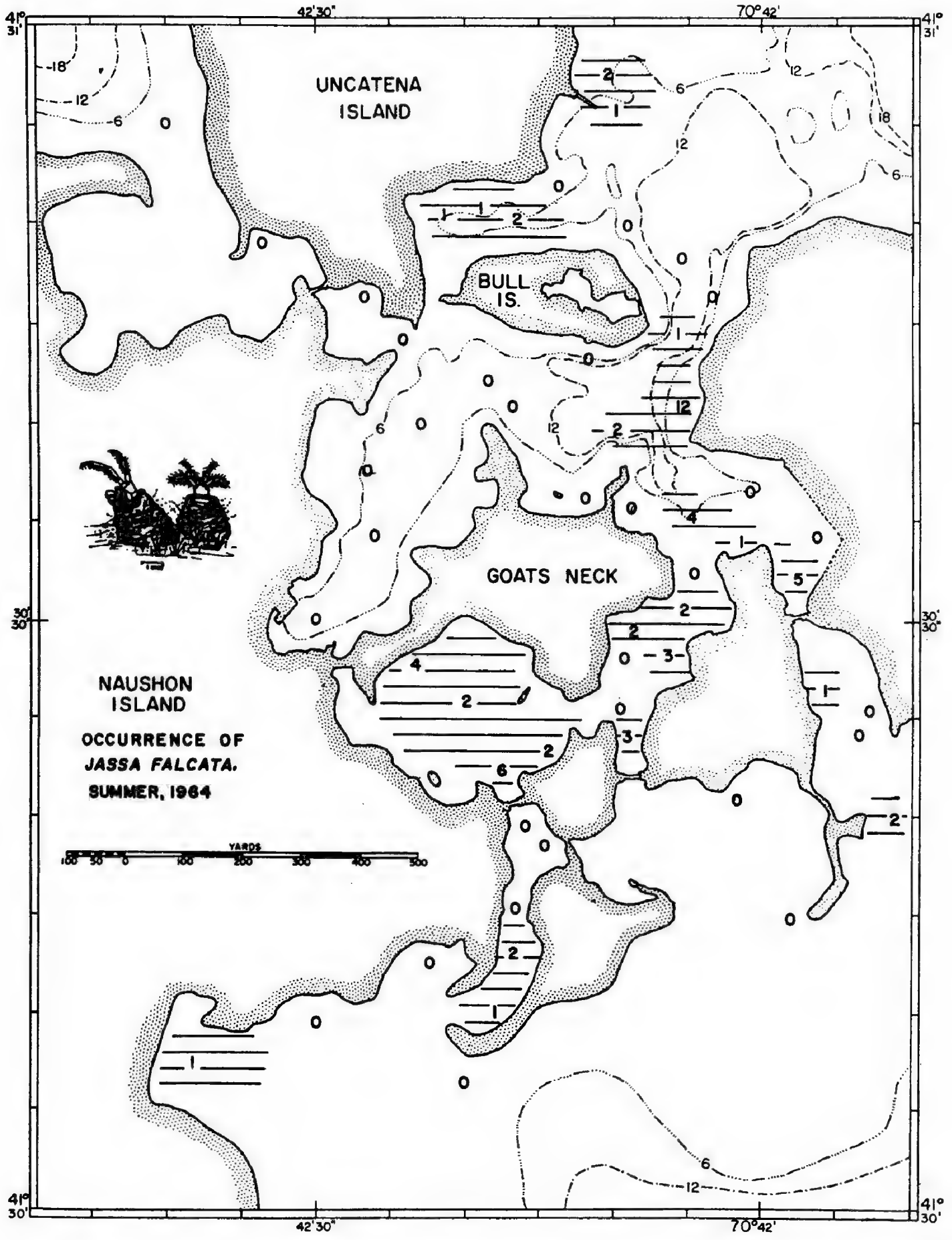

FIG. 8. Occurrence of *Jassa falcata* in summer of 1964. The insert depicts feeding position of this species; its strongly setose antennae make an efficient filtering mechanism.

areas of abundance, where populations show an aggregate distribution. Internal population characteristics vary from areas of presence to areas of abundance, as depicted in Figure 14 (representative of nearly all individual per station plots, taken each month). The areas of abundance appear to be favorable to the develop-

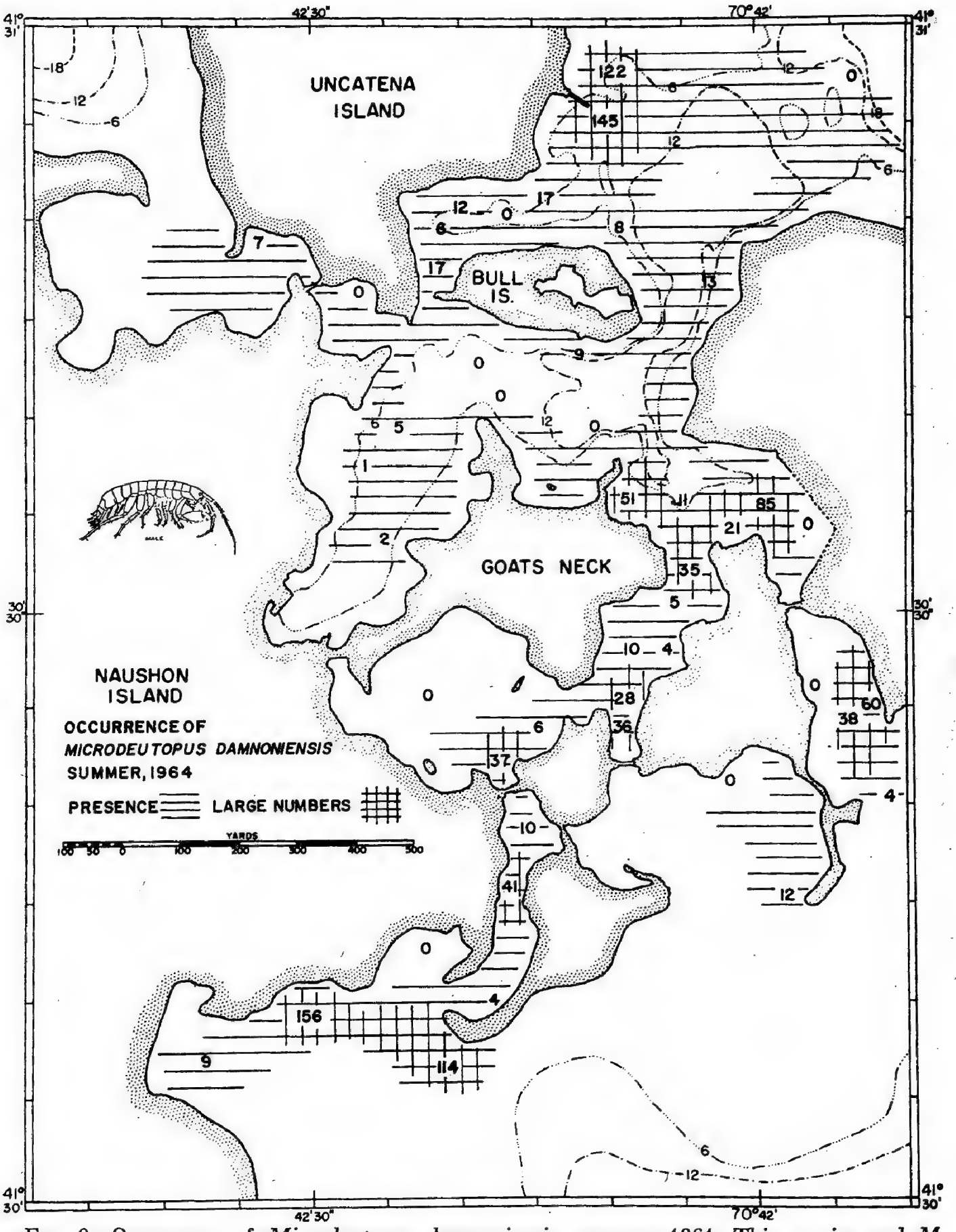

FIG. 9. Occurrence of *Microdeutopus damnoniensis*, summer 1964. This species and *M. gryllotalpa* (Fig. 10) occupy very similar ecological niches, yet their ranges overlap to a great extent. An explanation of their co-occurrence is offered in the text.

ment of large populations; at almost any month, one can find significantly (to 95%) higher numbers of ovigerous females, eggs per ovigerous females, and growing juveniles. Furthermore, in favorable areas several species of the same feeding type may cohabit the same portion of one plant. The areas of abundance are termed "favorable" areas, in that conditions suit the development of large popu-

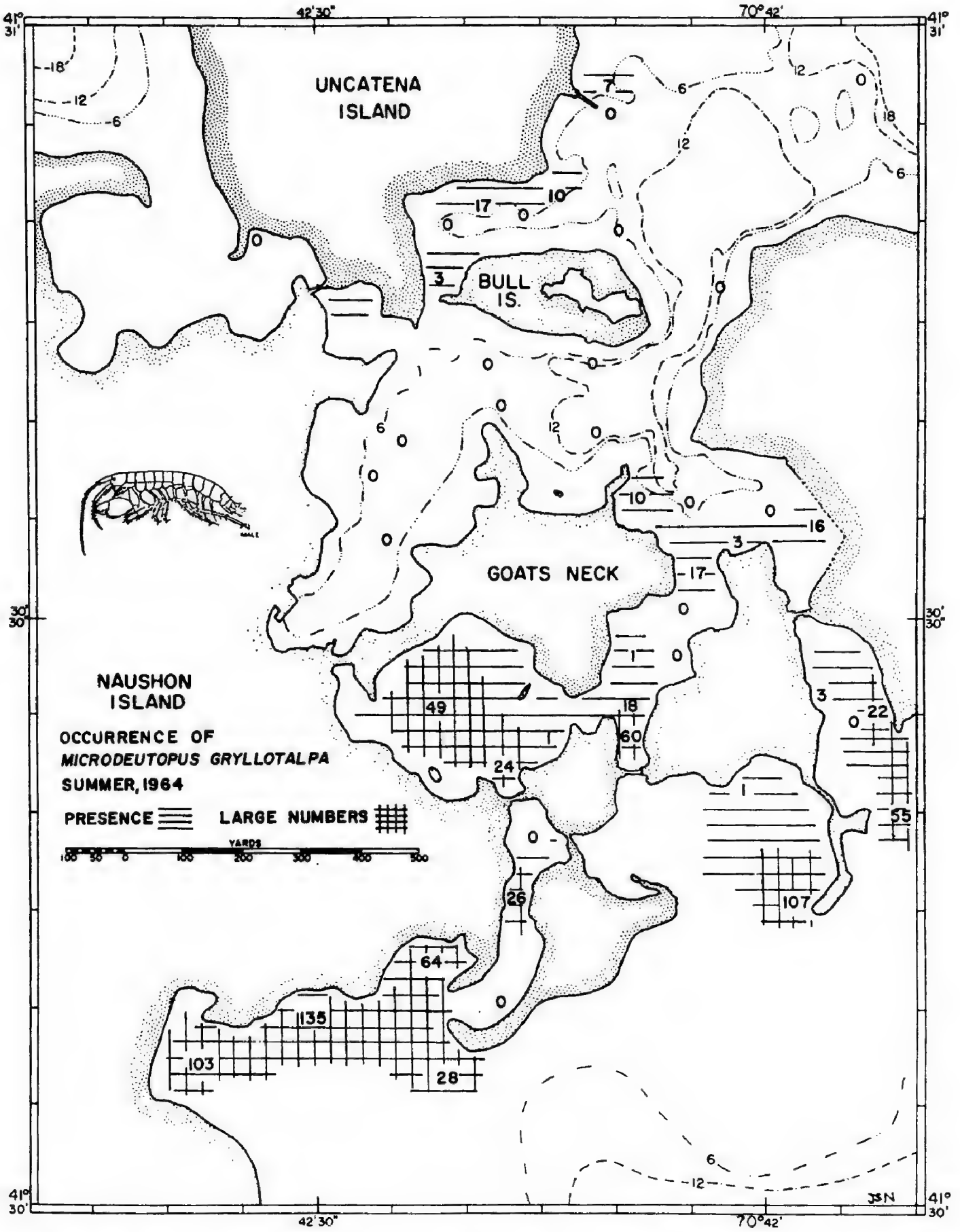

FIG. 10. Occurrence of *Microdeutopus gryllotalpa*, summer 1964.

lations and are probably near the physical or biological optima for the species. Areas of presence are termed "habitable" areas, the physical or biological parameters of these areas falling within the tolerance of the species, but such that large populations cannot develop. This concept of favorable and habitable areas is applied in this paper to analyses of physical and nutritional requirements of species populations within one major habitat; it is not applied to the ecotypic vari-

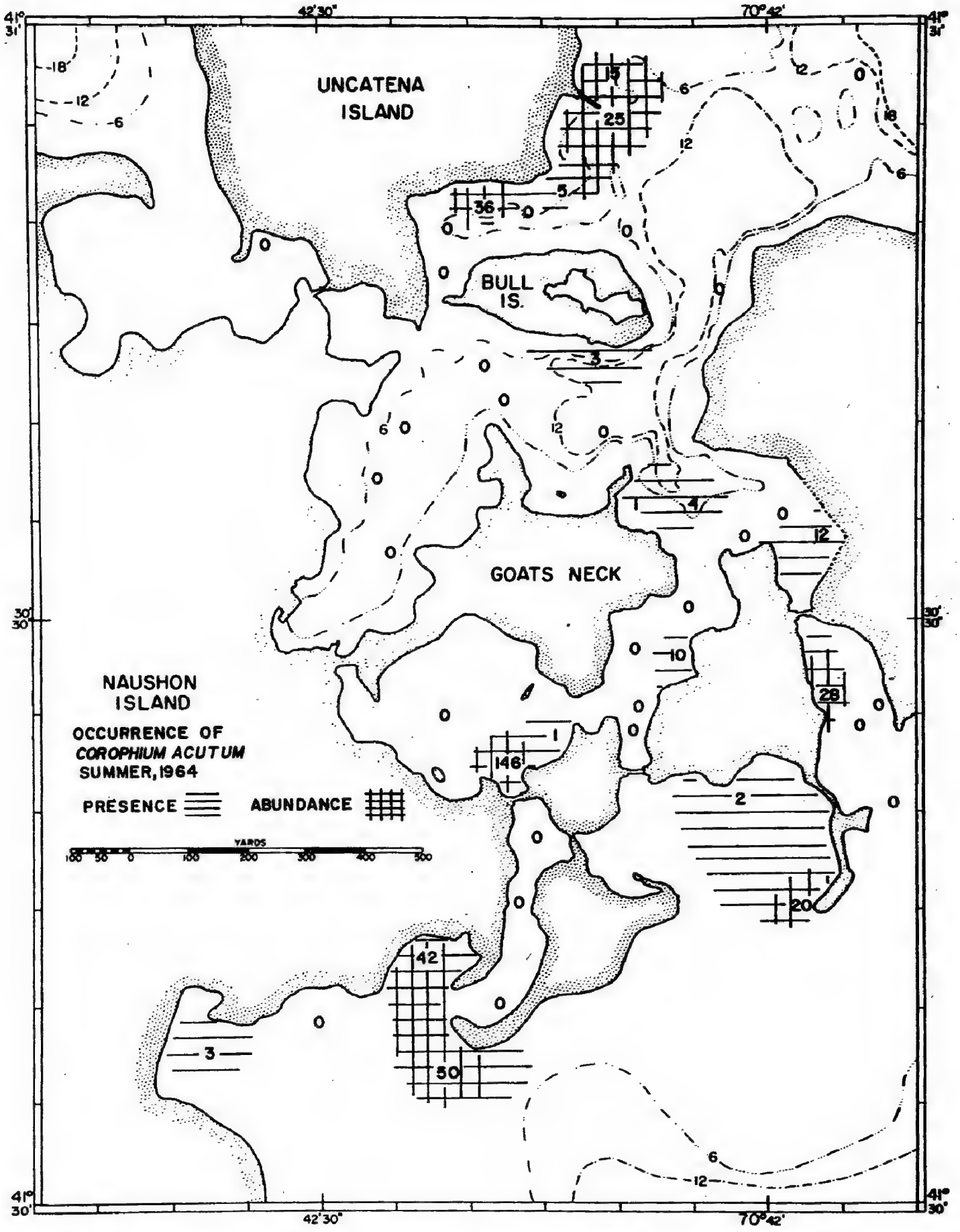

FIG. 11. Occurrence of *Corophium acutum*, summer 1964. This species and *C. insidiosum* (Fig. 12) occupy very similar ecological niches, yet their ranges overlap to a great extent. An explanation of their co-occurrence is offered in the text.

ants found over the geographic range of the species. If local aggregations are not density favored, an analysis of favorable versus habitable areas may elucidate physical optima for a species, as shortly exemplified for *Zostera*.

The peripheral separation of *Microdeutopus* and *Corophium* within the detri-

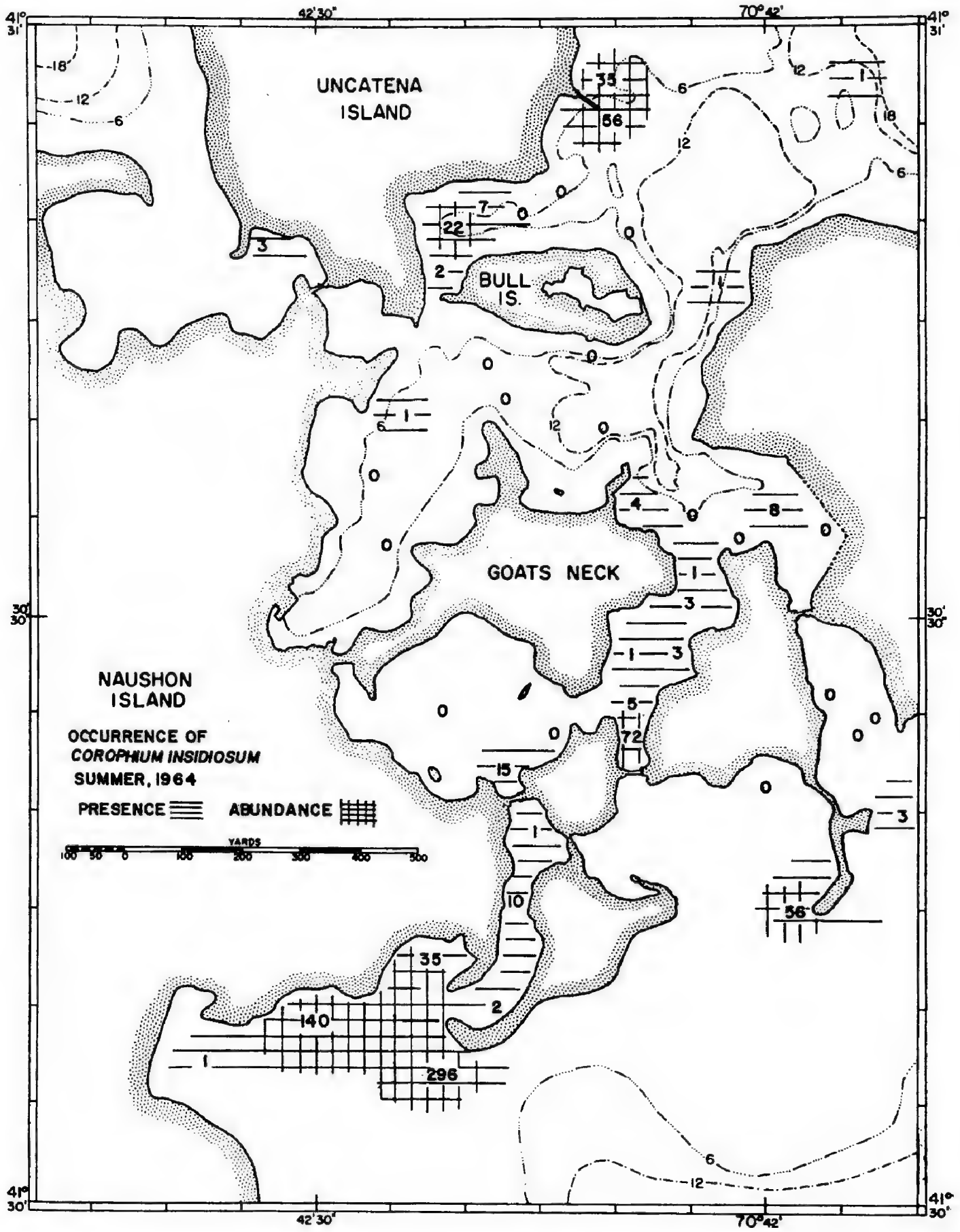

FIG. 12. Occurrence of *Corophium insidiosum*, summer 1964.

tus feeding group has been discussed before; this separation takes place only when long epiphytes are present. When epiphytes are relatively small, the two genera bodily compete for the same living space. In some habitats, *Microdeutopus* is able to displace *Corophium*; in others the converse is true. Experimentation elucidates the role of adaptive morphology in this competitive situation.

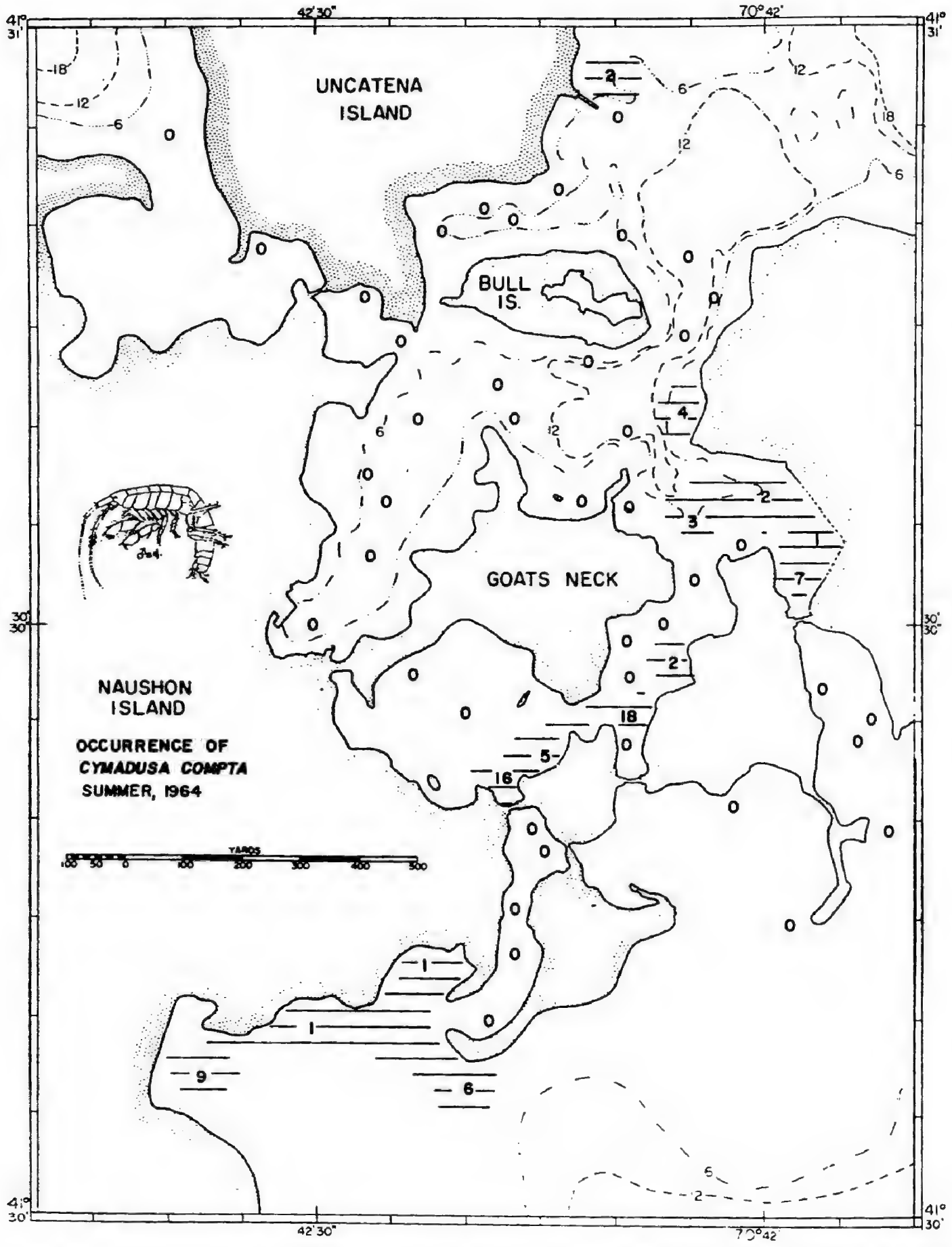

FIG. 13. Occurrence of *Cymadusa compta*, summer 1964. This species is restricted to areas with strong currents in normal marine salinity. It abounds in estuaries, where it replaces the ecologically similar species of *Microdeutopus*.

In the experiment, 10 each of adult *Microdeutopus damnoniensis* and *Corophium acutum* were placed in a finger bowl containing a 2 cm piece of epiphyte-bearing eelgrass that was clipped to the side of the bowl. First the water was left quiet and the number of each species not on the grass noted. Then the water was

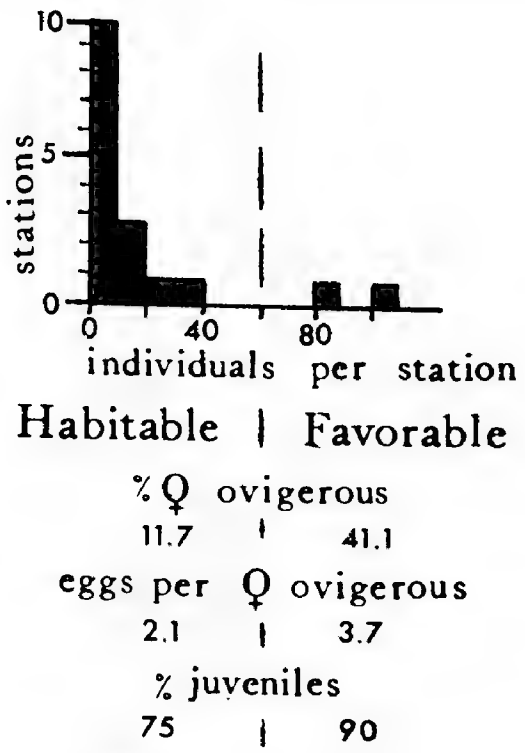

Fig. 14. Comparison of intraspecific population characteristics in habitable and favorable areas. *Microdeutopus damnoniensis* in October 1964 is illustrated, but the trends would be the same for all common species of amphipods in all summer and early autumn months.

circulated around the bowl at 20 cm per second by a magnetic stirrer, and the number of displaced members of each species noted. Amphipod behavior was noted during the experiment; results are tabulated in Table 4.

In quiet water, the larger *Microdeutopus* is able to pinch *Corophium* with its antennae, driving the smaller form away, but in moving water, *Corophium* is able to turn the tables on *Microdeutopus*. *Microdeutopus* is larger, strongly laterally compressed, and has relatively short 5th pereiopods; in currents, it struggles to hang onto the plant and can be easily dislodged by the smaller *Corophium* which is dorsoventrally compressed and which has longer 5th pereiopods. It presents a lower outline to the current, and its long 5th pereiopods render it more stable; hence it is able to maneuver freely over the grass during current flow. Under these conditions, it can evict *Microdeutopus*.

By the adaptive mechanisms discussed above, the genera *Microdeutopus* and

Table 4

Results of displacement experiment

Sample	Displaced microdeutopus	Displaced corophium	Time after start of experiment
	quiet water		
1	1	5	10 minutes
2	0	4	
1	2	6	1 hour
2	2	8	
	Current, 20 cm per second		
1	7	2	1 hour
2	8	4	

Corophium can either coexist on the same plant by occupying different physical spaces or displace the other when living space is not available. Hence, the spatial ranges of the genera do not overlap. But what of the species within each genus? If one compares the summer distribution of the species pairs, *Microdeutopus damnoniensis—M. gryllotalpa* (Figs. 9 and 10) and *Corophium acutum—C. insidiosum* (Figs. 11 and 12), one finds that their ranges strongly overlap, especially in areas of abundance. The range of *Corophium bonelli*, not illustrated, also coincides with that of the other species. It is not surprising to note that favorable areas for the amphipods correspond to favorable areas for *Zostera*; the amphipods must spend at least part of the year in favorable eelgrass areas, so should be adapted to the habitat. Differences in areal distribution are largely confined to habitable areas, occupied by *Zostera* and the amphipods only in summer. Here, amphipod ranges generally do not overlap, and give clues to the physical factors limiting certain species. *Microdeutopus damnoniensis* is restricted from the warmer portions of the shallow bays in summer; thermal tolerance experiments supported the field observations by revealing that the mean thermal death temperature of this species is 22 C, whereas that of *M. gryllotalpa* is 33 C. Habitable ranges of *Corophium insidiosum* and *C. acutum* did not overlap; no field or laboratory observations could account for this. Another important aspect of areal summer distribution of these species is not the slight difference in occurrence, but the strong coincidence. Observation of species on individual plants demonstrated that both members of these species pairs can inhabit the same portion of a plant. Differences in feeding habits are negligible. Nevertheless, these species pairs show no tendencies to competitive exclusion. Seasonal studies can partly explain their coexistence.

Cymadusa compta (Fig. 13) is never very abundant, and is restricted to highest physical energy areas, either wave dominant, as in Lackey's Bay, or current dominated, as in the gutters. *Cymadusa* is rare for two reasons: (1) it is at the northern limit of its range, which extends at least to Aransas Bay, Texas, and (2) it is an estuarine form, especially abundant in the Patuxent Estuary (Nagle 1965). Other euryhaline detritus-feeders include *Gammarus mucronatus, G. tigrinus,* and *Ampithoe valida.* The detritus-feeding species of *Gammarus* replace *Microdeutopus* in euryhaline waters, and the algal-feeding *Ampithoe valida* replaces *A. longimana* (and *A. rubricata*).

Distribution and form of the marine diatom-feeders, *Dexamine thea, Ampithoe longimana,* and *Leptochelia savignyi* are also correlative with energy. The large, flat-sided *Ampithoe longimana,* inhabits the gutters and protected parts of Lackey's Bay; it is unable to withstand the high energy of the open coasts. *Dexamine thea,* a much smaller, less compressed type, prefers the open coasts of Lackey's Bay and Uncatena Island, although small numbers can be found in the gutters. The squat, elongate tanaid *Leptochelia savignyi* attains peak abundance only on the outer, wave-swept coasts of Lackey's Bay, Uncatena Island, and in the constrictions of the gutters where currents may reach 5 knots. Its low profile appears to be especially well adapted to high energy situations.

Snails also show morphologic adaptations to energy regime. *Bittium alternatum,* a moderately high spired detritus feeder, is exceedingly abundant in the

gutters and current dominated parts of the harbor where its streamlined form presents low resistance to currents. Its place on the outer, wave-swept coasts is taken by *Lacuna pallidula*, whose nearly globose geometry presents the least surface area to the constant buffeting of the waves.

Bryozoan form also corresponds to energy regime. The crustose, *Membranipora*-like bryozoa and the semi-crustose *Hippothoa hyalina* inhabit the wave-beaten areas, whereas the ramose *Bugula* reaches peak abundance in current dominated grass beds.

Most of the plant dwelling epizoans are not restricted to a particular species of plant, or, as discussed previously, some are not even restricted to plants. Because of this independence of particular plants, the epizoans could survive the eelgrass blight of the 1930's and 1940's. The blight resulted in virtual decimation of the plant populations, but animals characteristic of the "eelgrass fauna" persisted (Dexter 1947). Furthermore, many of the algae show season fluctuations in abundance, completely disappearing in parts of the year (Conover 1958). It would be difficult for the perennially abundant epizoans to be restricted to such algae. Some animals, however, are restricted to perennially persistent seaweeds. The amphipod *Ampithoe rubricata* hides in the dense thalli of *Fucus*, its yellow-spotted olive body mimicking its background very closely. *Hyale nilssoni* is another amphipod that prefers algae, its light green color blending with *Enteromorpha*, its host plant. Other algaphyllous amphipods include *Marinogammarus* and *Calliopius*.

Many of the rarer members of the epibiota show interspecific population dependence on the commoner forms; for example, predatory turbellarians follow harpacticoid copepods. The bryozoan *Crisia eburnea* is a commensal upon the shell of *Bittium*; its occurrence reflects that of the host. Similarly, the tiny amphipods *Parametopella cypris* and *P. minuta* follow the distribution of hydroids, from which they steal food. Neither are abundant in Hadley Harbor. Chironomid larvae, rare in marine waters, increase with decreasing estuarine salinity behind Tobey's Island and in the Pocassett Estuary.

SEASONAL VARIATIONS

Breeding cycles

A puzzling aspect of *Corophium* and *Microdeutopus* populations appeared during the summer of 1964. At this time, it was possible to find large numbers of adults of both *Microdeutopus damnoniensis* and *M. gryllotalpa*, or *Corophium acutum* and *C. insidiosum* at any given locality. However, most of the juveniles belonged to only one of the species. At first, it was felt that juveniles had been misidentified, but careful re-examination of all specimens negated that possibility. Replicate samples at these stations verified the original observation that most of the juveniles did belong to only one species. A seasonal synthesis of population structure resolved the problem and provided a possible mechanism for coexistence of two species.

Graphic depictions of seasonal population changes in *Microdeutopus* and *Corophium* appear in Figures 15 and 16 respectively. Average abundance per month

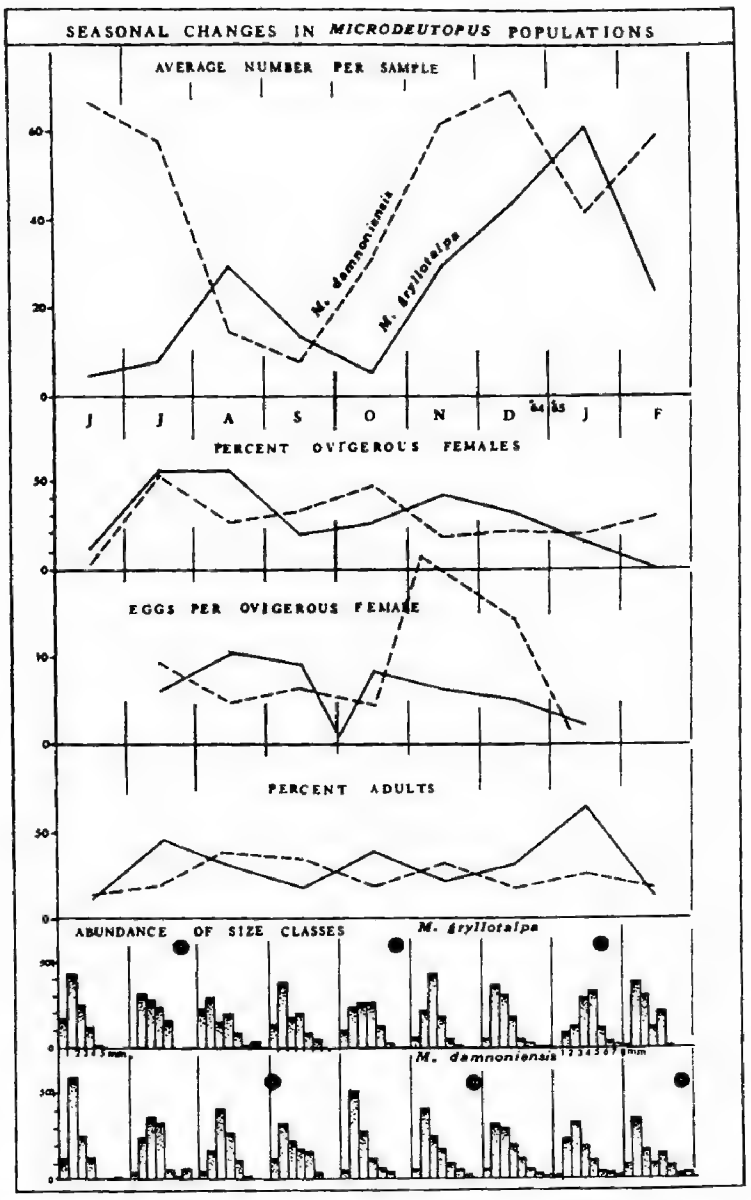

FIG. 15. Seasonal changes in *Microdeutopus* populations. The dark circles indicate dates of maximum juvenile release by ovigerous females; all trends point to staggered breeding cycles.

fluctuates wildly; some of this is probably related to not sampling at the same station every time, but fluctuations which follow breeding cycles probably do reflect population changes to some extent. Most of the conclusions concerning breeding cycles were not drawn from abundance, but from study of internal population characteristics, including size distribution, adult to juvenile ratios, percentage of ovigerous females, and number of eggs per ovigerous female. These changes were internally consistent through all the stations, reflecting population developments through the area, not vagaries in sampling.

Breeding patterns of *Microdeutopus* and *Corophium* differ from those of the scarcer animals; instead of breeding once a year, the amphipods exhibit almost continuous sexual activity. It is possible to find mating pairs and ovigerous females the year round. However, the amphipods do show peaks in fecundity; these are the basis for reconstructing breeding cycles.

Fecundity of *Microdeutopus gryllotalpa* peaks in July, October–November and January (Fig. 15). Great numbers of juveniles from the July release swell the population in August, as evidenced by the increase in young juveniles, 0 to 1 mm long, in that month. The summer brood matures in late October and November, after which there is another relative increase in juveniles. These juveniles mature

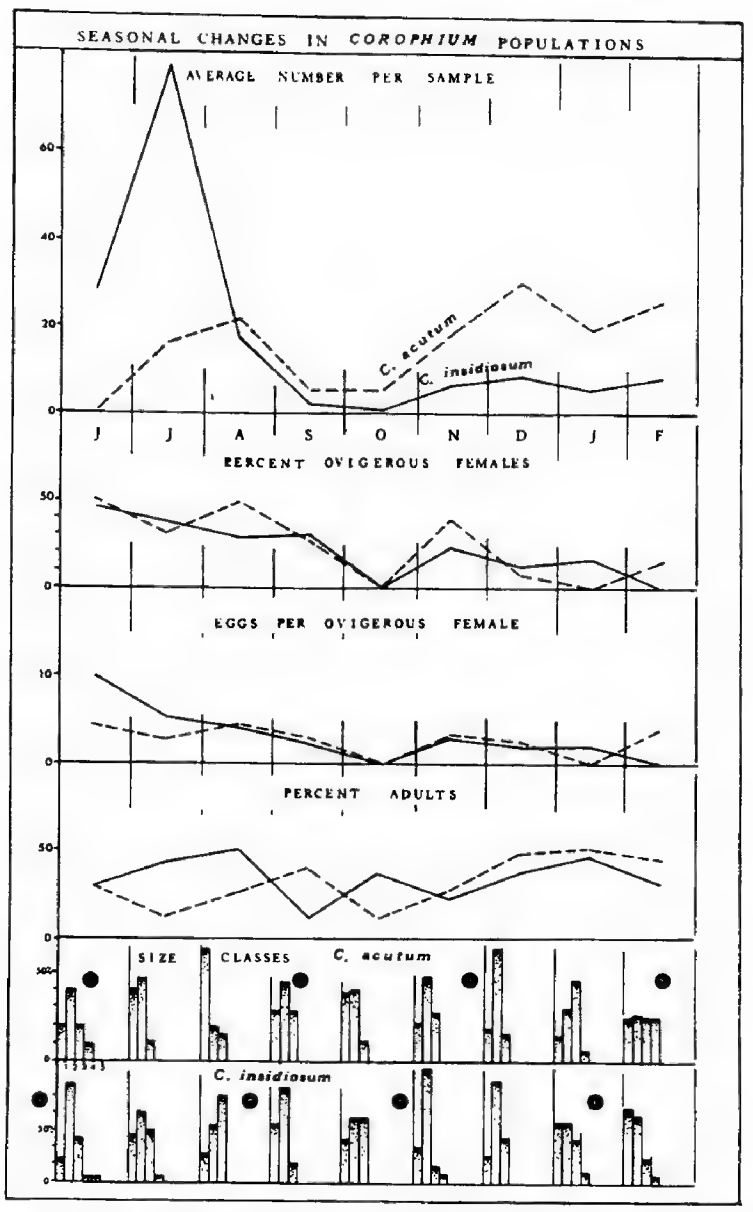

FIG. 16. Seasonal changes in *Corophium* populations. The dark circles indicate dates of maximum juvenile release by ovigerous females. All trends point to staggered breeding cycles.

and breed again by January. Adults of the summer generation are smaller, breeding at 3–4 mm, whereas winter forms carry eggs at a minimum size of 4–5 mm. Winter generations do not breed as actively as summer generations, as evidenced by the lower percentage of ovigerous females, which carry fewer eggs.

Microdeutopus damnoniensis exhibits breeding maxima in late August through early September, and in late November and early December. Spring-hatched juveniles, relatively abundant in June, gradually mature to 3–4 mm long summer adults in August. In October there is a flood of juveniles which attain the 5–7 mm winter adult size in late November and December and carry up to 22 eggs per female. The juveniles released at this time mature and breed by late February.

Breeding patterns of *Corophium* (Fig. 16) resemble those of *Microdeutopus*. *Corophium acutum* breeds in June, September, late November–December and February. June remnants of winter generations, 3 to 5 mm long, have a high percentage of ovigerous females which carry a relatively high number of eggs per female. These females are responsible for the flood of juveniles in July and August. The summer generation matures, breeds and releases its young by the end of September. The autumn generation seems to exhibit high mortality, probably coincident with the loss of its preferred substratum when *Zostera* dies down

for the winter; the late fall maximum does not exhibit the same dispersal as the earlier maxima, and will be explained shortly. Adults of the autumn generation breed in November and early December; their progeny mature and produce late in February. Some females may brood as they mature; it is possible to find ovigerous females as small as 3 mm and as large as 5 mm long in one sample. This is interpreted to indicate females having several broods during maturation.

Breeding maxima of *Corophium insidiosum* occur in August, late October, and January; the flood of juveniles in June and July is probably from a May brood. Percentage of adults decreases markedly after each brood and tends to be greatest when populations are at a minimum, a reflection of population attrition with growth. Neither species of *Corophium* breeds in mid-October when the eelgrass is undergoing a marked reduction in abundance.

The most interesting aspect of the breeding patterns of these species, however, is not the October minimum, but the alternation of cycles. Notice the alternations in abundance, percentage of adults, percentage of ovigerous females, periods of juvenile release and distribution of size classes (Figs. 15 and 16). Instead of overlapping, the breeding periods tend to be staggered. This alternation of breeding periods probably permits sympatry of otherwise incompatable species by reducing competition during the early juvenile stage. In this way, the areal distribution of these species pairs can coincide in favorable areas. Species-individual cumulative curves for these summertime communities appear to show a random distribution of species. A random distribution is characteristic of an ecologically stable situation (Sanders 1965), supporting the idea that the sympatric association is stable; one species is not being competitively excluded. Wintertime curves show less of a random distribution; the populations at that time appear to be unstable. Staggering of breeding cycles parallels that of penaeid shrimp (Williams 1955); summer niche diversity parallels that of intertidal haustoriid amphipods (Croker 1967), which show slightly different behavior and substratum preferences, even though their spatial ranges overlap greatly. Interspecific fighting between *Corophium* and *Microdeutopus* resembles that of two competing species of beach hoppers (Bower 1964). The consequence of such interaction is the spatial separation of populations.

Other members of the epizoa, such as the amphipods *Ampithoe*, *Cymadusa*, the temporary resident ostracods *Parasterope* and *Propontocypris*, and the gastropods *Bittium* and *Lacuna* have only one brood per year. Their populations exhibit the usual increase in juveniles just after mating season and a gradual decrease in numbers coincident with an increase in size until maturity is attained. Most of these animals overwinter in an adult or advanced juvenile stage. Areal distribution of these forms do not overlap; their breeding cycles are not staggered as are those of the sympatric amphipods. Breeding in copepods was not observed, but it may take place as many as 7 times in a season (Wilson 1932).

Seasonal migrations

The other seasonal aspect considered here is the change in distribution of the eelgrass epibiota as a whole, especially the changes related to the winter defoliation of the grass in habitable areas. Favorable areas for *Zostera* are shallow and highly

agitated (Fig. 6A). In addition to obtaining nourishment from the soil, the plant appears to be capable of obtaining nutrients from the water column (Conover, personal communication, 1965). Its occurrence in Hadley Harbor supports these experimental observations; it is most abundant where water exchange, either by currents or wave agitation, is greatest. *Zostera* must have abundant sunlight to effect this nutrient exchange, or it will not prosper; it is relatively scarce in the current-swept, but deep portions of the gutters. In summer, when the sun is high overhead, sunlight can penetrate through the murky waters of the deep gutters, enough for a sparse eelgrass cover to develop. Some plant growth also takes place in summer in the relatively quiet shallows of the lagoons, but such areas are only habitable to the plant, and it never flourishes. In summer, the epibiota colonizes *Zostera* in the habitable areas. In October and November, *Zostera* undergoes a marked decline in biomass, as graphically depicted by Conover (1958, p. 113). This decline is not uniform; in favorable areas the plant remains relatively abundant all year (Figs. 17 and 18), but in habitable areas it completely defoliates, leaving its epibiota without a substratum.

Comparison of epizoal dispersion in summer and winter gives a clue to what happens to the epizoa. In summer (Fig. 17) the fauna is abundant wherever there is grass, although there are never extremely great numbers of animals at one place. The average is 360 per station, or only 9000 per square meter. In winter (Fig. 18) there are relatively more stations with a depauperate epifauna, but there are also a number of stations with a very abundant epifauna, averaging 1500 per station, 37,500 per square meter. The great abundance of epizoa coincides with the places where the grass remains high. Comparison of faunal trends in selected localities reveals the changes in faunal dispersion.

Summer, autumn and winter stations in the deep part of the East Gutter, between Bull and Nonamessett Islands, show relatively little change in infaunal abundance, but the epifauna decreases markedly in autumn and winter (Fig. 19), coincident with the defoliation of the grass. And stations in the quieter portions of the Northwest Gutter from June, July, October and December, 1964, and January, 1965, show a similar pattern (Fig. 20A). The major decrease in faunal abundance corresponds to the autumn defoliation of *Zostera*.

The opposite pattern holds in favorable areas for the grass. In the shallower portions of East and Southwest Gutters, the epifauna is abundant, but remains at a relatively low level through September, reflecting the summer dispersal of epizoa. It more than doubles in October, before decreasing to a relatively high level in winter (Fig. 20B). And in the favorable shallows of Outer Harbor, October and November see a spectacular increase in the epifauna, followed by a drop-off in numbers (Fig. 20C).

The explanation of these faunal changes seems clear. Population declines in habitable areas for *Zostera* correspond to population increases in favorable areas. Overall epifaunal population for the Hadley Harbor complex increases slightly, from an average of 360 epizoa per sample to 430 (although the 430 individuals per sample come from fewer areas). Population changes come at the time *Zostera* is defoliating. Apparently the motile members of the epifauna migrate from the dying eelgrass to the areas where it remains viable. The substratum-preference ex-

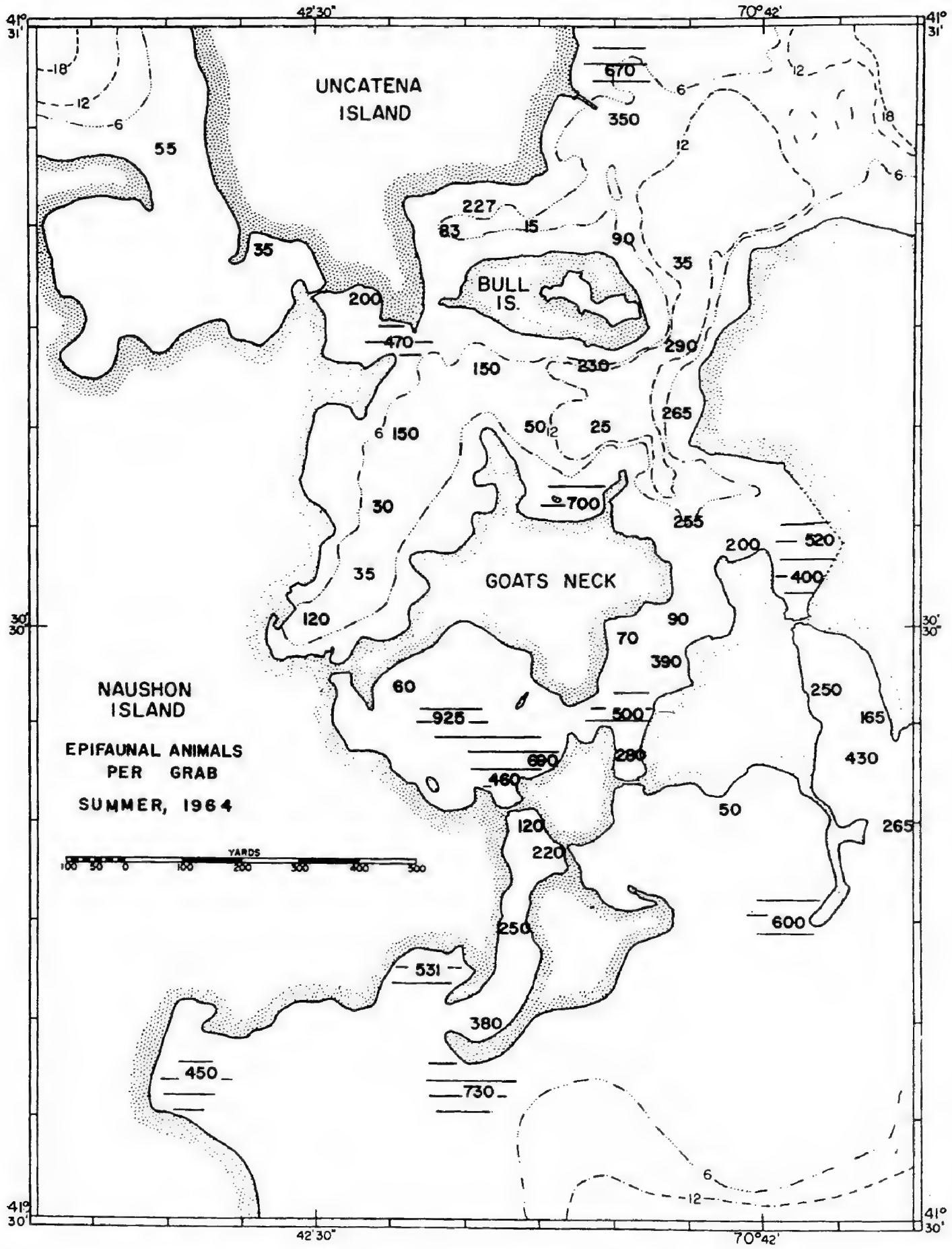

FIG. 17. Epifaunal animals per grab, summer 1964. Compare with Fig. 18. Summer populations tend to be more dispersed.

periments demonstrated that the animals have the capability of locating their preferred substratum; the mechanism for migration exists. Average increase in epizoa per station probably reflects migrations from deeper waters outside the Hadley Harbor complex. After the populations have migrated, competition presumably

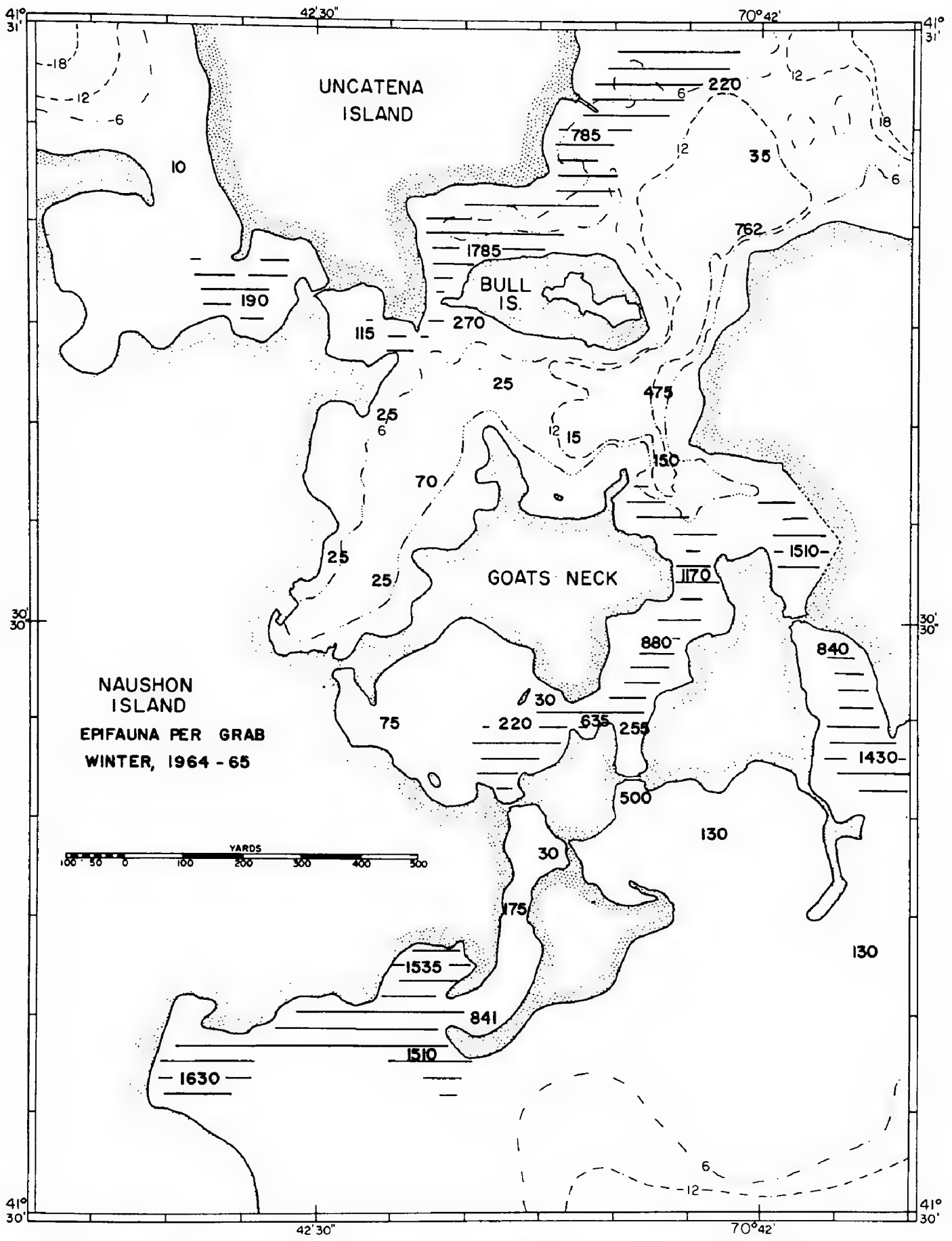

FIG. 18. Epifaunal animals per grab, winter 1964–65. Extremely abundant animals occur where eelgrass remains high all year.

reduces them to a lower level, still higher than in summer. Autumn increases in *Microdeutopus* and *Corophium* populations, then, are not a result of breeding cycles, but of faunal migration. Their wintertime populations are restricted to areas of abundant *Zostera*.

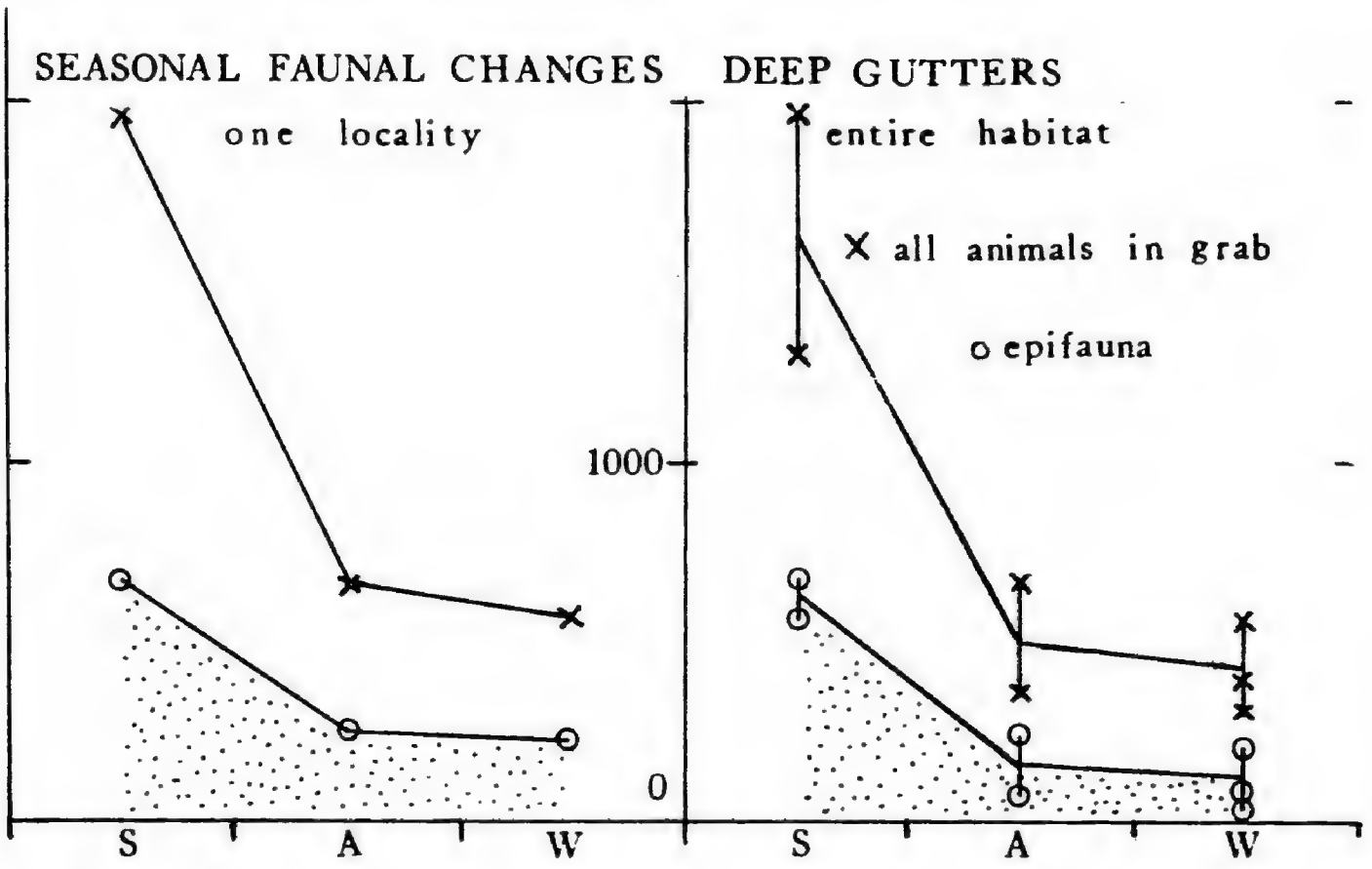

FIG. 19. Seasonal faunal changes, deep gutters. Epifaunal trends are emphasized by the stippled pattern; the autumnal decline corresponds to the defoliation of *Zostera* in these areas.

In this fluctuating environment, no species can become closely adapted to a particular physical regime. The epibiota disperses with the spring flowering of *Zostera*, as evidenced by the relatively even distribution of summer populations (Fig. 18). Populations can stabilize and diversify to slightly different habitats in summer, as indicated by differences in occurrence and thermal tolerances in *Microdeutopus*. However, epizoa cannot become too well adapted to these summer habitats because they are removed with the autumn defoliation of *Zostera*. The epizoans are forced to the favorable areas for eelgrass, and these have a limited range of physical conditions. Thus, epifaunal animals must be adapted to both summer and winter conditions; they cannot specialize to a particular habitat. Implications of this will be considered shortly.

PARALLELISM

The concept of the parallel benthic community, as presented by Thorson (1957) can be applied to the epibiota of macroepibenthic plants as well as to level bottom communities. Samples from the Patuxent Estuary of the Chesapeake Bay, Vellerup-Vig-Isefjord, Denmark, and Texas Coast localities near Port Aransas illustrate this point. Most of the samples from the Patuxent Estuary were collected by Joseph Mihursky, Hallowing Point Field Station of the Chesapeake Biological Laboratory, Prince Frederick, Maryland, with supplementary collections and observations by the author in September, 1965 (Nagle 1965). Samples from Denmark were collected by R. H. Parker in summer, 1965. Texas Coast samples, collected in April and August, 1966, came from the jetties at Port Aransas, Harbor Island turnaround one mile north of Port Aransas, the lighthouse channel and

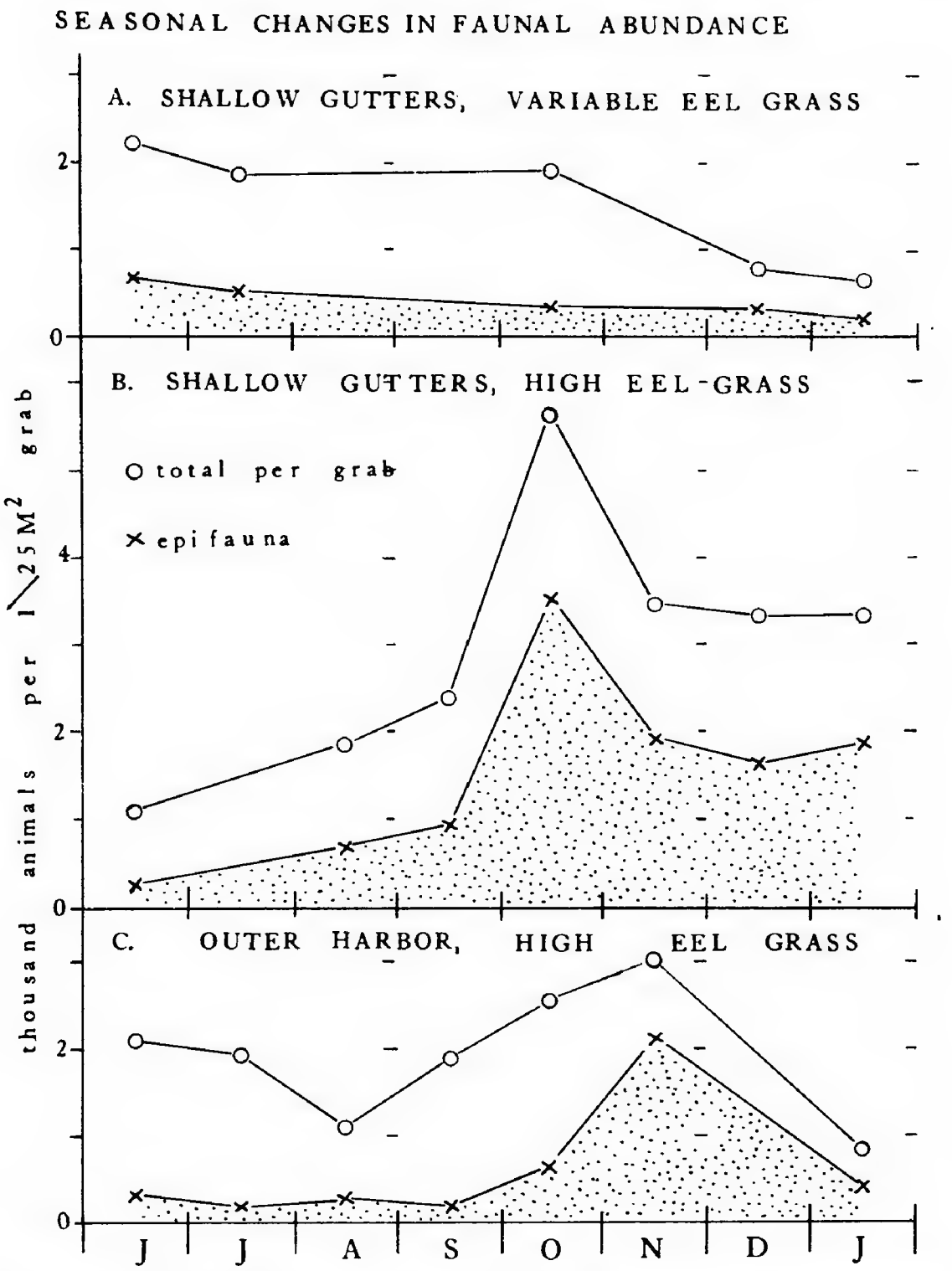

FIG. 20. Seasonal changes in faunal abundance. Epifaunal trends are emphasized by the stippled pattern. The epifaunal increase in areas where the grass remains high corresponds to a decrease in areas where the grass defoliates.

Quarantine Shore of Harbor Island, *Diplanthera* shoals at the swing bridge between Corpus Christi and Padre Island, and at Indian Point on the confluence of Corpus Christi and Nueces Bays.

It was observed or inferred that general trophic-abundance trends are the same, although diversity varies with environment. Detritus cleaners predominate everywhere, with six abundant species on Cape Cod, four in the moderately saline waters of the Isefjord, four in the euryhaline waters of the Texas Coast, and only two in the lowered-salinity estuarine waters of the Patuxent. Foulers and diatom grazers were less abundant, with three species in the Isefjord, one in the Patuxent Estuary and Aransas Bay. Parallel taxa in these areas are shown in Table 5.

TABLE 5

Parallel members of macroepibenthic plant epibiota, as found in this study

Cape Cod	Isefjord	Patuxent Estuary	Texas Coast
	Detritus feeding amphipods		
	Fam. Aoridae		
Microdeutopus gryllotalpa	*M. gryllotalpa*	.....	*Microdeutopus* n. sp.
M. damnoniensis	.....	.....	*Rudilemboides* n. sp.
	Fam. Corophiidae		
Corophium acutum	*Erichthonius difformis*	*Corophium lacustre*	.....
C. insidiosum	*C. insidiosum*	.....	.....
C. bonelli	*C. bonelli*	.....	.....
	Fam. Ampithoidae		
Cymadusa compta	.....	*C. compta*	*C. compta*
	Fam. Gammaridae		
Gammarus mucronatus	*Gammarus zaddechi*	*G. mucronatus*	.....
G. tigrinus	.....	*Melita nitida*	*M. nitida*
G. annulatus	.....	.....	.....
	Detritus feeding gastropods		
Bittium alternatum	*B. alternatum*	.....	*Bittium varium*
Lacuna pallidula	*L. pallidula*	.....	.....
	Diatom feeding amphipods and tanaid		
Leptochelia savignyi	.....	*Leptochelia* sp.	*Leptochelia* sp.
Dexamine thea	*Dexamine spinosa*	.....	.....
Ampithoe longimana	.....	.....	.....
	Suspension feeding amphipods		
Caprella geometrica	*Phtisica marina*	.....	.....
Paracaprella pusilla	.....	.....	.....
Jassa falcata	.....	.....	.....
	Algal-dwelling amphipods		
Calliopius laeviusculus	*C. laeviusculus*	.....	.....
Hyale nilssoni	.....	.....	*Hyale* sp.
	Climbing ostracods		
Loxoconcha guttata	*L. guttata*	*L. guttata*	*L. guttata*
Cytherura spp.	*Cytherura* spp.	.....	*Cytherura* spp.
Cytherois spp.	*Cytherois* spp.	.....	*Cytherois* spp.
Hirschmannia viridis	*H. viridis*	.....	.....

Cymadusa compta dominates the detritus-scavenging amphipod fauna in both Chesapeake and the Texas bays; *Grammarus* and *Melita* are present, but rarer. Other common detritus scavengers in Texas include new species of *Microdeutopus* and *Rudilemboides*. *Gammarus zaddechi*, *Erichthonius difformis*, and *Microdeutopus gryllotalpa* abound in the Isefjord. In Denmark, the caprellid *Phtisica marina* parallels the Cape Cod *Caprella* and *Paracaprella*. *Bittium alternatum* is the common detritus feeding snail on Cape Cod and in the Isefjord; its place on *Thallasia* of Aransas Bay is taken by *Bittium varium*. The Danish diatom feeding amphipod *Dexamine spinosa* parallels, in ecological position, *Leptochelia savignyi*,

Dexamine thea, and *Ampithose longimana* of Cape Cod. An intermediate species of *Leptochelia* occupies this ecologic station in the Texas bays and the Patuxent estuary; its broad range of habitats illustrates the generalization of estuarine forms. In the Patuxent, *Leptochelia* not only inhabits seasonally fluctuating plants, but also occupies intertidal sands, where it takes the place of as many as 10 species of haustoriid amphipods of more saline coasts.

The estuarine epifauna is dominated by pericarid malacostracans, even more than the onshore marine epifauna of Cape Cod. Female pericarida carry the eggs and young until they have reached a fully developed juvenile stage. Animals which hold the young past the larval stage tend to occur in physically unstable areas (Thorson 1957, p. 479). This tendency increases in macroplant epifauna, and reaches a maximum in estuaries, which are very unstable. The increase in oviviparity in unstable areas probably reflects adaptation to an unstable physical regime, in which it is advantageous for animals to shift habitat at any stage in life. Planktonic larvae, at the mercy of water masses, do not have this capability and are probably less capable of survival in an unstable environment than are freely-motile juveniles. *Calliopius laeviusculus* prefers *Fucus* clumps in both Cape Cod and the Isefjord, whereas *Hyale nilssoni* and a parallel species of *Hyale* occupy *Enteromorpha* beds in Cape Cod and Texas. The ostracods *Cytherura*, *Cytherois*, and *Loxoconcha* crawl about on plants in Cape Cod and the Texas bays; they abound, and probably behave the same, in Danish *Zostera* patches.

GAUSE'S PRINCIPLE AND DIVERSITY OF EPIZOANS

The interspecific competition studies of Gause (1934) have been interpreted by a number of authors to state, in general, that competitively exclusive interaction occurs in a stable community when two or more niches overlap in any essential component that is in short supply. Since 1934, many field and laboratory studies have supported this principle by revealing niche-separating differences in allied organisms. Many recent studies, cited earlier, have elucidated mechanisms by which animals could exist sympatrically.

Niche separation in Hadley Harbor takes several forms. Field occurrence of potentially competing detritus feeding snails, diatom feeding amphipods, and suspension feeding amphipods reveals niche separation very simply—the animals' spatial distributions do not overlap. Except for the snails, these forms are relatively rare, probably because of limited food supply rather than living space or physical parameters. Niche separation in the abundant detritus feeding amphipods is less clear; the animals and their food supply abound in Hadley Harbor. Seasonal evidence indicates that the different species occupy more niches in summer than in winter. (Niche diversity is considered in terms of feeding type, peripheral distribution on long epiphytes and areal relationships to physical parameters). In summer, members of the *Microdeutopus* species-pair exhibit different habitable areas, with *M. damnoniensis* prevalent in quieter waters and some level bottoms but excluded from the warm shallows where *M. gryllotalpa* prevails. Habitable areas of the *Corophium* species-pair and *C. bonelli* do not overlap in summer, although the reason is unexplained. The fact remains that

species within a peripheral distribution type show slight differences in spatial distribution in summer, and at least some of these differences are adaptive and serve to delimit niches. In winter, areal ranges of all species of epizoal amphipods coincide with almost complete overlap because the animals are restricted to the favorable areas for *Zostera*. Such areas have a limited range of physical parameters. Hence all species of a particular feeding and peripheral distribution type must occupy the one physical regime available in winter. Even the apparent staggering of breeding cycles in summer becomes less clear in the winter. One should expect competition for food to be limiting in winter; but the increased supply of organic detritus resulting from decomposition of defoliated *Zostera* can provide a greater food supply to the winter communities, allowing for closer spacing of individuals.

Marine niche separation has been determined for tropical gastropods (Kohn 1959), temperate intertidal polychaetes (Mangum 1964), nematodes (King 1962) and amphipods (Croker 1967). The habitats for these animals are relatively stable all year, especially for the tropical gastropods which show a great number of small niche differences. In stable situations, marine communities show diversity rather than dominance (Sanders 1965). Summertime community structure (when determined by the method of Sanders) shows greater stability than wintertime structure; this appears to be correlative with summer niche diversification. It is probable that Gause's Principle does not apply well to wintertime epizoal communities because they are occupying an unstable situation; studies in stable communities, cited above, follow Gause's Principle reasonably well.

The combination of niche separation and unity is probably the best adaptation to a community that fluctuates so widely from season to season. Such generalized adaptations to physical instability are not necessary in stable areas such as the deep sea or tropics; there one finds very diverse communities (Sanders 1965) and many very closely related species with slightly different physical and nutritional requirements (Kohn 1959). Whittaker (1965, p. 257) offers four hypotheses to account for species diversity in different plant communities. Of these, only the first seems applicable to the epibiota of macroepibenthic plants, "severe, unstable, and recent environments limit the numbers of species which have evolved to maintain themselves in those environments. In older and less severe environments larger numbers of species tolerate environmental conditions and maintain themselves with finer differentiation of their environmental requirements." Sanders has independently restricted that hypothesis, postulating that biological interaction in physically stable areas results in increased diversity; interaction with the physical environment, rather than biological interaction, results in reduced diversity in physically unstable areas. The concept of niche separation and unity, set forth above, provides a mechanism for reducing diversity in response to physical instability.

SUMMARY

1. Although the epibiota of macroepibenthic plants shows many local variants, the more or less permanent epizoa show three distribution patterns on individual plants. They can (1) decrease exponentially upwards on the stem, (2) increase

upwards on the stem and (3) vary with epiphytes. Those that decrease upward are primarily bottom dwellers whose populations spill over onto plants. The taxa that increase upward are primarily suspension feeding fouling organisms which must live high up in the water column to obtain required nutrients. Forms that vary with epiphytes either graze on the epiphytes or protocooperatively interact with the epiphytes to gain detritus food, protection from predators, and material for domicile construction. The epiphytes benefit from being cleaned by the epizoans, for if not cleaned, they would become coated with detritus and die. However, animals can live independently if food and protection are present, and epiphytes can exist independently if strong waves and currents keep them detritus-free. Motile epizoans locate epiphytes, their preferred substratum, by sight and touch, rather than by distant chemoreception.

In addition to a vertical zonation on macroplants, animals show a peripheral zonation on long epiphytes. Snails and predominantly domicolous amphipods of the genus *Corophium*, forms relatively immune to fish predation, live near the tips of the epiphytes. More motile amphipods such as *Microdeutopus*, which are susceptible to fish predation, live near the bases of the epiphytes.

2. Summer amphipod populations in a locally varied habitat show both dispersion and concentration. Areas of random dispersion are termed habitable areas; physical and biological factors fall within the species' tolerance but apparently are not near the optima for development of large populations because there are fewer ovigerous females which carry fewer eggs, yielding lower percentages of juveniles. Habitable areas for similar feeding and peripheral distribution types show little spatial overlap. Areas of amphipod concentration are termed favorable areas; fecundity here is much higher than in habitable areas, and physical and biological conditions are probably near optimal. Ranges of species competing for the same food and living space can coincide in such areas, apparently occupying the same ecological niche.

Caprellid amphipods, suspension feeders that prefer high energy, occur in wave-swept bays and current dominated channels; the animals are morphologically well adapted to their habitat. *Jassa falcata*, a lower energy suspension feeder, has setose antennae which form a net to effectively fish the quieter waters of its preferred habitat. Diatom feeders show a close correspondence of form to habitat, with the squat, elongated tanaid *Leptochelia savignyi* inhabiting the highest energy areas; the small, moderately compressed amphipod *Dexamine thea* inhabiting lower energy areas, and the large, compressed amphipod *Ampithoe longimana* occupying the lowest energy areas. Ranges of these species show little overlap. The relationship of form to energy noted above was also found in parallel communities and may be of general applicability.

Favorable areas for detritus-cleaning and amphipods, *Microdeutopus* and *Corophium*, generally coincide with areas of high physical energy, with *Corophium* showing a significantly better adaptation to currents. Habitable areas for two species in these genera do not overlap, *Microdeutopus damnoniensis* being excluded from areas of high summer temperature and *M. gryllotalpa* from quiet waters. Ranges of *Corophium* species in habitable areas do not overlap, but differences in adaptation are not clear.

3. The rarer epizoans breed but once a year, and overwinter as adults or late

stage juveniles. Detritus-feeding amphipods breed throughout the year, but their breeding cycles are staggered. This staggering, in addition to slightly different habitat preference, may allow these competing species to coexist and can explain niche differentiation in summer. Spatial ranges of these potentially competing species are forced to coincide in autumn and winter because eelgrass, the commonest preferred macroplant, defoliates in habitable areas. The plant remains high all year only in favorable areas which have high annual luminosity and water exchange. Great concentrations of epizoans on eelgrass leaves, coupled with virtual absence of epifauna in areas of winter defoliation, indicates the fauna migrates to the areas where the grass is perennially abundant.

4. The parallel community concept can be applied to the epibiota of macroepibenthic plants as well as to infaunal communities. Parallel species of genera, occupying similar trophic positions, in similar relative abundance, occur in Denmark, Cape Cod, the Patuxent Estuary, Maryland, and Texas bays. In these areas, pericarid malacostracans predominate in proportion to increasing environmental instability. The pericarids' habit of retaining young until they have attained a freely-motile juvenile stage appears to be an adaptation to physical instability. Newly-hatched young as well as adults are capable of shifting habitat, presumably to escape intolerable physical conditions.

5. Summer-dispersed populations of amphipod species-pairs occupy slightly different habitats and show some adaptational differences to these habitats. Summer communities tend to be stable; competing species do not co-occur where food and living space are scarce. Hence, summer populations follow Gause's Principle of competitive exclusion. Winter communities tend to be unstable, and competing species follow Gause's Principle less closely. Two or more species appear to occupy the same niche at this time; similar feeding and peripheral residence types are forced into the same physical regime by the defoliation of eelgrass in habitable areas. The species may occupy different physical regimes in summer, but are restricted to the one winter regime where *Zostera* remains abundant. Competition in winter is probably strong, but decomposing defoliated *Zostera* probably supplies the detritus necessary to maintain several species in the same niche, at least temporarily.

An epizoal species must be adapted to this physically unstable habitat; it must be adapted to its dispersed summer areas and to the one winter habitat available to all epizoa. This dual adaptation is believed to be a mechanism for reducing species diversity in an unstable environment. Species cannot diversify by developing finer differentiation of physical and biological requirements, as they do in physically stable areas.

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A NEW SPECIES OF CARANGID FISH (*DECAPTERUS TABL*) FROM THE WESTERN ATLANTIC[1]

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ABSTRACT

A new species of *Decapterus* is described from off the coast of Colombia, Venezuela and Florida. It is differentiated from the two other western Atlantic species of *Decapterus* by the numbers of scales in the curved lateral line, scutes in the straight lateral line, and total scales and scutes (61–78 scales, 34–48 scutes, 103–119 total in the new species; 37–56 scales, 32–46 scutes, 77–98 total in *D. punctatus*; 68–79 scales, 23–32 scutes, 119–133 total in *D. macarellus*).

INTRODUCTION

The carangid genus *Decapterus* occurs in most tropical and subtropical marine waters of the world. Several of the species, at least, occur in large pelagic schools. These are generally found in inshore waters, although the schools of young, especially, may occur in oceanic areas. Some species are utilized for human food, and some species are caught for live or processed bait for other fishes.

My recent studies, based upon a large amount of recently collected material, as well as specimens preserved in a number of museums, indicate that the species composition of *Decapterus* is inadequately known and even the morphological limits of the genus need critical study. The genus *Decapterus* was established by

[1] Contribution No. 78, Tropical Atlantic Biological Laboratory.

Bleeker in 1851 with the type species *Caranx kurra* Cuvier, 1833. Species of the genus were dealt with by Nichols (1911, 1936, 1951). Eastern Atlantic species were considered by Norman (1935), Trewavas (1945), Blanc and Bauchot (1961) and Cadenat and Marchal (1963). Western Atlantic species were described by Ginsburg (1952). The most recent review of the entire genus, by Bertin and Dollfus (1948), is in need of revision.

Recent literature (Ginsburg 1952; Briggs 1958; Berry in McClane 1965) has defined only two species of *Decapterus* from the western Atlantic—*Decapterus punctatus* (Agassiz in Spix 1829) and *Decapterus macarellus* (Cuvier in Cuvier and Valenciennes 1833). The names of these two species have been considered, recently (Blanc and Bauchot 1961; Cadenat and Marchal 1963), to apply to species in the eastern Atlantic. A third species from the eastern Atlantic, with a confusing nomenclatorial history, has been referred to by the name of *Decapterus sanctaehelenae* (Cuvier in Cuvier and Valenciennes 1833) by Cadenat and Marchal (1963); and in their check list, Jordan, Evermann and Clark (1930) used that name to apply to a supposed third species from the western Atlantic. My current review of this genus in the western Atlantic is benefited from a large and significant amount of specimen material collected in the last few years by the research vessels OREGON, COMBAT, SILVER BAY, CALAMAR and by James K. Dooley. This has been supplemented with recent collections from the eastern Atlantic by the LA RAFELLE and THIERRY of the Guinean Trawling Survey and the UNDAUNTED, GERONIMO and PILLSBURY. These studies have produced the following conclusions:

1) Three species of *Decapterus* occur in the western Atlantic, *D. punctatus*, *D. macarellus*, and a new species described herein.
2) Cognate (or geminate) forms, or species of these, occur in the eastern Atlantic.
3) The scientific name of the eastern Atlantic cognate species of *D. punctatus* is *D. sanctaehelenae* (Cuvier in Cuvier and Valenciennes 1833).
4) *D. macarellus* occurs on both sides of the Atlantic.
5) The new species described here from western Atlantic material also occurs at St. Helena in the eastern Atlantic.
6) Four species of *Decapterus* occur at St. Helena, and one of these (the "stonebrass") apparently lacks a valid scientific name.
7) *Caranx rhonchus* Geoffrey St. Hilaire, 1809, has recently been placed in the genus *Caranx*, but is a valid species of *Decapterus*.
8) Indo-Pacific species of *Decapterus* are in need of revision, and although they appear to be specifically distinct from Atlantic species, at least one may represent a circumtropical species complex (including *D. macarellus*).

CHARACTERS AND METHODS

The methods of taking accurate data are frequently dependent on knowledge of morphology and ontogeny of the characters, and the 2 topics are combined here. This section relates primarily to the 3 western Atlantic species of *Decapterus*, but the several *Decapterus* species I have examined from other areas con-

form to these characters and methods, and for most of them, so do species of *Selar*, *Trachurus*, and other Caranginae. Only western Atlantic specimens (excluding Bermuda) are used in the species diagnoses and in the tables (except for 2 St. Helena specimens in Table 5).

Dorsal fin. The dorsal fin is continuous in small specimens; the membrane connecting the 8th and 9th dorsal spines exists to a size of *ca.* 30–50 mm SL. The first dorsal fin normally has 8 spines (one aberrant specimen had only 4); the third spine is normally the longest, and the longest spine is *ca.* 10–40% longer than the longest softray. The second dorsal fin has one anterior spine (that is *ca.* one-half as long as the longest softray) and 29–37 branched softrays; the first softray is normally the longest and *ca.* 10–28% longer than the longest anal fin softray; the terminal finlet in *punctatus* is close to the penultimate softray in small specimens (*ca.* less than 18 mm SL), and becomes displaced posteriorly at larger sizes; the membrane connecting the finlet to the penultimate softray degenerates at *ca.* 45–50 mm SL.

Anal fin. The anal fin is continuous in small specimens; the membrane connecting the second and third spines exists to *ca.* 30–40 mm SL. The first anal fin has 2 spines, with the first the slenderer but usually *ca.* 3–12% longer than the second. The second anal fin has one anterior spine (*ca.* one-half as long as the longest softray) and 24–31 branched softrays; the first softray is normally the longest; ontogeny of the finlet is similar to that in the dorsal fin.

Lengths of dorsal and anal rays. Spines and softrays measured from anterior point of insertion to tip of ray.

Pectoral fin. Each pectoral fin has one spine on its dorsal margin (*ca.* one-fifth to one-third as long as the longest softray) and 18–23 softrays; the first softray is unbranched, the others branched; the fourth softray is the longest, with the third only slightly shorter. Bilateral variation occurs in the number of softrays (27% of the sample of *tabl* had one more softray on one side than the other, 20% of *punctatus*, and 8% of *macarellus*), but the variation is random, either right or left sides having the extra ray. The pectoral fin is *ca.* 45–80% longer than the pelvic fin.

Pelvic fin. Each pelvic fin has one lateral spine (*ca.* one-half to two-thirds as long as the longest softray) and 5 branched softrays (one abnormal specimen had only 4 softrays in the right fin); the second softray is the longest with the first only slightly shorter.

Lengths of pectoral and pelvic fins. Measured from the dorsal (pectoral) or lateral (pelvic) point of insertion of the spine to the tip of the longest ray.

Caudal fin. In *Decapterus*, as in all Carangidae, there are 9 dorsal (superior) and 8 ventral (inferior) principal caudal fin rays, with the most dorsal and the most ventral ray unbranched and the others branched; there are *ca.* 8–9 secondary caudal rays in both the dorsal and ventral lobes. The caudal fin rays are all considered to be softrays, even though the 3–4 most anterior dorsal and ventral

secondary rays are not segmented. The most ventral of the dorsal principal rays is usually the shortest ray in the fin; the longest ray in the dorsal lobe is the third from the top principal ray, and the corresponding ray in the ventral lobe is its longest; the dorsal and ventral lobes are of similar length.

Gillrakers. Gillrakers occur on both the lateral and medial sides of arches 1–4 (numbered from lateral to medial); gillrakers on the lateral side of the first arch are about 3–8 times as long and almost twice as numerous as on the medial side of the first arch; the gillrakers on faces of the other arches generally decrease slightly in number (toward the fourth arch), with the medial side of the fourth arch lacking upper limb gillrakers and having the lowest count.

The count of gillrakers on the lateral side of the first arch is used in the text, in characterizing the species. The gillraker at the angle of the upper and lower limb has the major portion of its base over the ceratobranchial bone and is included in the count of the lower limb. Occasionally, rudimentary gillrakers (with the base width of the raker equal to or greater than its length) occur at the anterior ends of the limbs, over the epibranchial and hypobranchial bones, and these are included in the counts for each limb. The number of gillrakers over the hypobranchial has a slight positive correlation with that over the ceratobranchial. Apparently, gillrakers continue to form on the anterior ends of the upper and lower limbs to a size of *ca.* 55 mm SL; specimens smaller than 60 mm SL were not included in the tables of frequency distributions for the 3 species.

A slight bilateral variation exists in gillraker numbers, that is randomly right or left. Counts were usually made only on the right side of each fish. It is often necessary to slightly damage the opercular and isthmal attachments and to use compressed air to adequately expose all of the gillrakers for counting.

Branchiostegal rays. Branchiostegal rays were 3 + 4 on both sides in all specimens counted (22 specimens of *tabl,* 18 of *punctatus,* and 10 of *macarellus*).

Vertebrae. Number of vertebral centra is intraspecifically very constant in all Carangidae. I have examined all genera and many species (some in appreciable quantity) of the family and find no variation in number within a species, with four exceptions: 1) One 106-mm specimen of *Decapterus sanctaehelenae* from St. Helena (ZMK uncataloged) has 10 + 14 centra, and 52 other specimens of the species have 10 + 15. 2) One *Oligoplites saurus inornatus* has 10 + 17 centra, and 19 others have 10 + 16. 3) One *Hemicaranx amblyrhynchus* has 10 + 15 centra with a deformity of the last centrum, and 15 others have 10 + 16. 4) The *Caranx sexfasciatus* species complex varies between 10 + 14 and 10 + 15 dependent on geographic area of occurrence. Although Bertin and Dollfus (1948:6) did not consider vertebral number to be of value in specific differentiation of *Decapterus*, my data show (with the one exception given above) that *tabl* and *macarellus* have 10 + 14 centra and *punctatus* and *sanctaehelenae* have 10 + 15.

Trunk vertebrae in *Decapterus* have a bilateral pair of pleural ribs on centra 3–10. Caudal vertebrae counts include the urostyle as the terminal centrum; the posterior surface of the first interhemal spine ankyloses to the anterior surface of the first hemal spine at a small size.

Scales. Body scales are cycloid with concentric circuli around the focus, and usually have one or two scallops on the anterior margin with two or three weak radii separating these and extending from the anterior margin to the focus. The body is covered with scales except for small areas in the axil of the pectoral fins and beneath each pelvic fin. Scales extend anteriorly on top of the head to above the anterior part of the eye and largely cover the interorbital area; they extend ventrally over most of the opercle, subopercle and interopercle; and from the anterior part of the preopercle scales extend onto the cheek to the end of the maxilla. The spinous dorsal and spinous anal fins are scaleless. Rows of scales occur on the membranes between the first three of four rays of the soft dorsal and soft anal fins. Rows of scales occur along the anterior margins of the softrays of the pectoral and pelvic fins and on the membranes between the rays of the caudal fin. There are no bilateral, paired caudal keels. The scaled sheath along the bases of the dorsal and anal fins is low. The posterodorsal accessory lateral line (supratemporal commissure) is relatively short, converging and ending on each side near the posterior end of the supraoccipital crest.

Lateral line. The lateral line in *Decapterus* and all Caranginae consists of an anterior curved (dorsally arched) part, that is marked by a single row of pored scales beginning at the cleithrum, and a posterior straight (horizontal) part, that has a single row of pored scales and scutes and ends on the median portion of the caudal fin. The "junction" of the curved and straight parts is defined as the point where (extending posteriorly) the lateral line ceases to bend and becomes straight and generally aligned with the horizontal body axis. It is not a precise point, and a somewhat arbitrary point must be selected in measuring and counting. The site of this junction varies within a species and frequently varies on each side of a fish, but when the degree of intraspecific variation is known, it can be a useful taxonomic character. A scute in carangids is defined as a modified pored lateral-line scale that is enlarged (compared to other body scales), usually thickened, and has its posterior margin terminating either in a pointed spine or an apex, with the angle of the scute formed by this margin being about 110° or less.

The total number of scales and scutes in the lateral line appears to be present in the smallest specimen counted for each species (*punctatus*, 29 mm; *tabl*, 65 mm; *macarellus*, 37 mm). The differentiation of scales to scutes is not complete at the smallest sizes in *punctatus* and *macarellus*, however. In *punctatus*, scutes tend to form into the posterior part of the straight lateral line until a size of about 48 mm, although this may be completed in some specimens as small as 30 mm. In *macarellus*, straight lateral-line scutes are apparently all formed in specimens 51 mm and larger, but not in 6 specimens of 37 to 50.5 mm. Data on these smaller specimens of *punctatus* and *macarellus* were not included in Table 4.

Lengths of curved and straight parts of lateral line. The curved lateral line was measured as a chord of its arc; the straight lateral line was measured from the junction to the end of the last scale over the caudal fin.

Ratio of lateral line. The lateral-line ratio is the quotient of the length of the straight part divided by that of the curved part.

Dentition. Bertin and Dollfus (1948:6) minimized the value of dentition in *Decapterus* taxonomy, while based on data of Suzuki (1962:215–216), differences in tooth patterns will differentiate six nominal species of *Decapterus* he identified from Japan. The dentition of the specimens of *tabl* is generally similar in pattern, but quite variable in number and extent—suggesting that both ontogenetic changes and intraspecific variation should be analyzed in describing dentition in other *Decapterus* species. Also, teeth are easily broken off, especially in the jaws, during laboratory examination. They are present in most specimens of the three western Atlantic species in both jaws, on the tongue and palatines, and laterally on the head and medially on the shaft of the vomer.

Body measurements. Standard length (SL)—tip of snout to end of caudal base (hypural bones) along horizontal body axis. Fork length (FL)—tip of snout to end of shortest median principal caudal fin ray. Total length (TL)—tip of snout, through horizontal body axis, to a vertical of tip of longest caudal fin ray. Head length—tip of snout to posterior margin of fleshy opercle. Eye diameter—horizontal diameter of orbit (often estimated and difficult to determine due to thickness of adipose eyelid). Postorbital head length—posterior margin of orbit to posterior margin of fleshy opercle. Upper jaw length—tip of snout to posterior margin of maxillary. Maxillary depth—greatest depth from dorsal margin of supramaxilla to ventral margin of maxilla. Body depth D1 to P2—origin of first dorsal fin to insertion of pelvic fin in saggital plane, the measurement inclined anteroventrally. Body depth D2 to A1—origin of second dorsal fin to origin of first spine of anal fin, the measurement inclined posteroventrally. Body depth maximum vertical—measured in saggital section, usually occurring around insertion of spines 4–7 of first dorsal fin.

KEY TO WESTERN ATLANTIC *DECAPTERUS*

1A. Dark spots (10–14) present along curved lateral line (at sizes larger than *ca.* 90 mm SL). Curved lateral line strongly arched (Fig. 4). Curved lateral line with 37–56 scales and usually 1–5 scutes. Total lateral line scales and scutes (excluding peduncle scales) 72–98. Vertebrae 10 + 15 ..... *Decapterus punctatus* (Fig. 2).

1B. No spots in curved lateral line. Curved lateral line moderately to weakly arched (Figs. 5 and 6). Scales in curved lateral line 61 or more. No scutes in curved lateral line. Total lateral line scales and scutes 103 or greater. Vertebrae 10 + 14 ..... 2.

2A. Curved lateral line moderately arched (Fig. 5). Anterior scales in straight lateral line 0–8. Scutes in straight lateral line 34–44 ..... *Decapterus tabl* (Fig. 1).

2B. Curved lateral line weakly arched (Fig. 6). Anterior scales in straight lateral line 19–33. Scutes in straight lateral line 23–32 ..... *Decapterus macarellus* (Fig. 3).

FIG. 1. *Decapterus tabl*, TABL 101476, 182 mm SL, OREGON station 4843, caught off Colombia, in a trawl fishing on bottom at 100 to 120 fathoms.

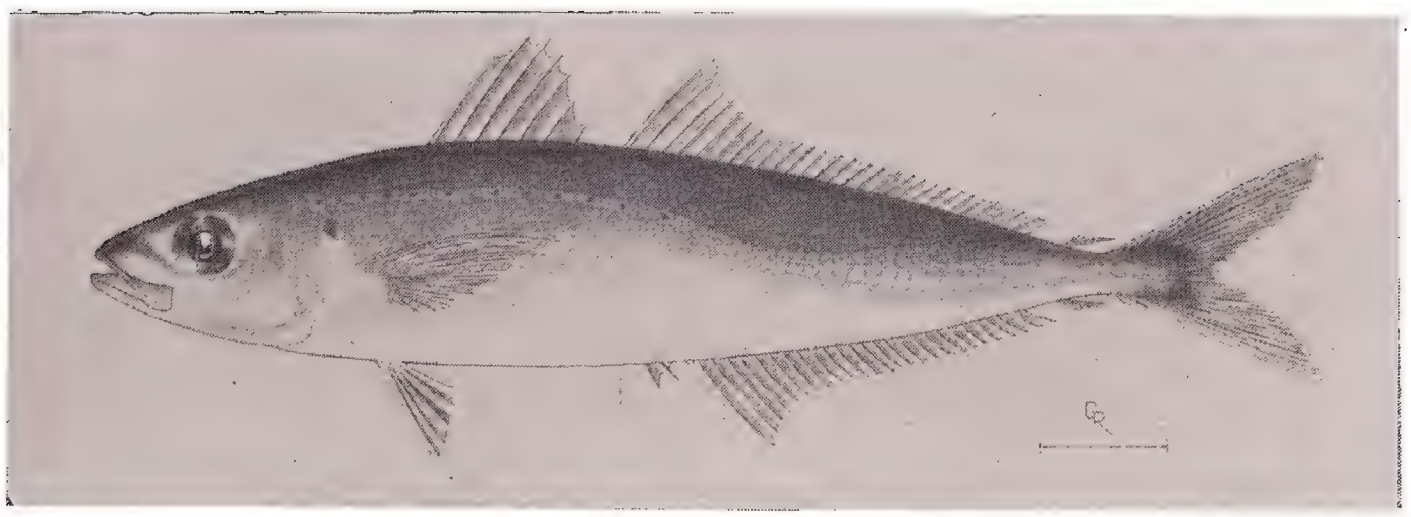

FIG. 2. *Decapterus punctatus*, TABL 101477, 159 mm SL, SILVER BAY station 5737, caught off North Carolina, in a trawl fishing on bottom at 25 to 29 fathoms.

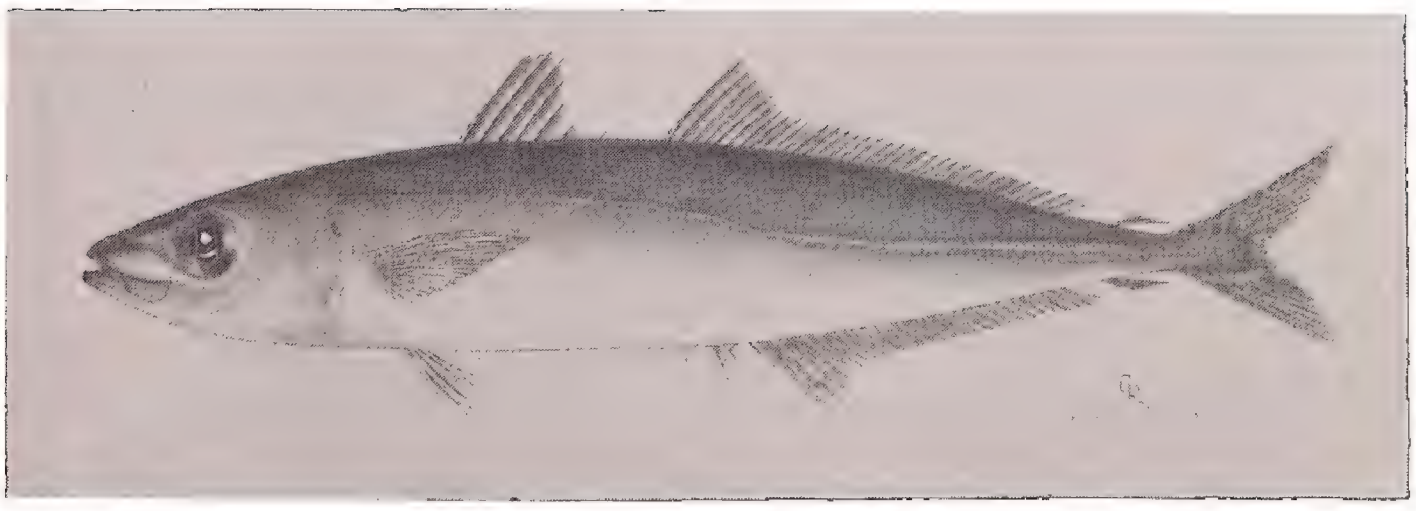

FIG. 3. *Decapterus macarellus*, USNM 84030, 172 mm SL, Woods Hole, Massachusetts.

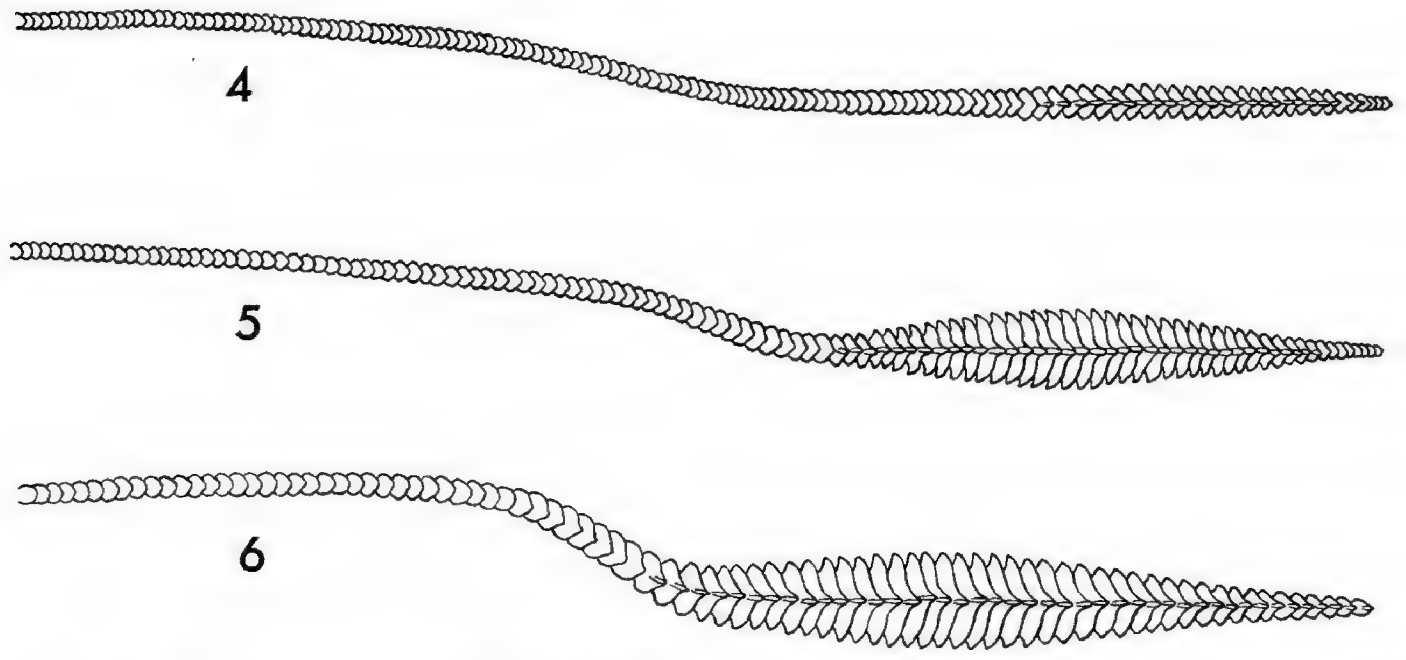

FIG. 4. *Decapterus macarellus*. FIG. 5. *Decapterus tabl*. FIG. 6. *Decapterus punctatus*. Contour of the lateral lines and relative size of their scales and scutes for the specimens of Figs. 1–3.

DECAPTERUS TABL, NEW SPECIES

REDTAIL SCAD

FIG. 1

Decapterus punctatus (*non* Cuvier): Ginsburg, 1952: 87 (in part; "the New Smyrna variant"). Berry in Anderson and Gehringer 1959: 64 (part of stomach contents of *Coryphaena hippurus*).

Diagnosis. A *Decapterus* with a relatively long curved lateral line, of 61–78 scales; no spots or scutes in curved lateral line and usually 0–8 scales in anterior part of straight lateral line; 34–48 scutes; lateral-line scales and scutes (excluding peduncle scales) 103–119; gillrakers 10–12 + 30–33; pectoral softrays 20–22; vertebrae 10 + 14.

Description. Frequency distributions of correlated dorsal and anal fin softray counts are given in Table 1. The dorsal and anal rays are positively correlated, with modal values of D. 31–32 and A. 25. Frequency distributions of correlated

TABLE 1

Frequency distribution correlation for dorsal and anal softrays in 50 specimens of *Decapterus tabl.*

		DORSAL					
		29	30	31	32	33	34
ANAL	27	..	..	..	..	2	2
	26	..	..	3	4	7	3
	25	1	1	10	11	2	..
	24	..	3	..	1	..	..

TABLE 2

Frequency distribution correlation for upper and lower limb gillrakers in 47 specimens of *Decapterus tabl.*

		LOWER			
		30	31	32	33
UPPER	12	..	4	4	2
	11	1	7	16	2
	10	1	6	4	..

numbers of upper and lower limb gillrakers on the lateral side of the first arch are given in Table 2. The upper and lower limb gillrakers are positively correlated, with a mode at 11 + 32. Frequency distributions of dorsal, anal, and pectoral softrays and of gillrakers are shown in Table 3 for comparison with the other two western Atlantic species. Gillrakers apparently had not completed formation in the smallest specimen (65 mm), and its count (9 + 30) is not included in Tables 2 and 3. Gillrakers over the hypobranchial range 9–11. One rudimentary gillraker was present at the anterior end of the upper limb of almost half of the specimens, and two rudiments were present in one specimen. Rudiments were lacking on the anterior end of the lower limb in more than two-thirds of the specimens; the others had one rudiment. The shape of the lateral line is illustrated in Figure 5. Frequency distributions of scales and scutes in various parts of the lateral line are given in Table 4. Scales in the curved and anterior parts of the lateral line (anterior to the first scute) range 65–78, mean 71.4. Posterior scales in the straight lateral line (on the peduncle and caudal fin, following the terminal scute) range 4–8, mean 6.0. Total scales in the lateral line (including the peduncle scales) range 110–124, mean 116.7. Vertebrae are consistently 10 + 14 in the holotype, 48 paratypes, and the three other specimens examined.

Ranges for significant morphometric characters of the larger specimens are given in Table 5, where paratypes of 156–199 mm SL from five collections are distinguished from the 14 larger paratypes of 195–219 mm from OREGON sta. 5711 and the two very large specimens from St. Helena. The larger paratypes, compared to the smaller ones, evidence a longer head, larger eye, longer pectoral and pelvic fins, and longer third spine and first softray in the dorsal fin. I presume these differences represent an allometric growth pattern in these characters. The St. Helena specimens are generally similar to *tabl* from the western Atlantic in body proportions.

The junction of the curved and straight parts of the lateral line is usually under the 13th to the 16th dorsal softray. The point of junction has a slight bilateral variation in most specimens, but the curved portion is up to 10% longer on one side than the other in a few specimens.

Dentition. All teeth small, short, conical, granular or deciduous, some slightly recurved. Teeth usually present in both jaws and on vomer, palatines, and tongue, but variable between otherwise similar specimens and with ontogeny. Upper jaw teeth in a short, irregular row near symphysis; lower jaw teeth in

TABLE 3

Frequency distributions of numbers of softrays in the dorsal, anal, and pectoral fins and of gillrakers in the three western Atlantic species of *Decapterus*

DORSAL SOFTRAYS

	29	30	31	32	33	34	35	36	37	$\bar{x}$	n
punctatus	2	8	18	29	12	5	..	..	..	31.8	74
tabl	1	4	14	16	11	5	..	..	..	31.9	51
macarellus	..	..	1	1	14	9	9	2	2	34.0	38

ANAL SOFTRAYS

	24	25	26	27	28	29	30	31	$\bar{x}$	n
punctatus	..	1	7	30	28	6	2	..	27.5	74
tabl	4	26	17	4	..	..	..	..	25.4	51
macarellus	..	..	..	2	9	18	7	2	28.9	38

PECTORAL SOFTRAYS (bilateral counts)

	$\frac{18}{18}$	$\frac{18}{19}$	$\frac{19}{19}$	$\frac{19}{20}$	$\frac{20}{20}$	$\frac{20}{21}$	$\frac{21}{21}$	$\frac{21}{22}$	$\frac{22}{22}$	$\frac{22}{23}$	$\frac{23}{23}$	$\bar{x}$	n
punctatus	13	10	35	4	7	..	..	..	..	..	..	18.9	69
tabl	..	..	..	..	4	8	32	6	1	..	..	20.9	51
macarellus	..	..	..	..	..	..	12	2	19	1	2	21.7	36

GILLRAKERS: UPPER LIMB

	9	10	11	12	13	14	15	16	$\bar{x}$	n
punctatus	..	..	5	13	61	49	12	2	13.4	142
tabl	..	11	26	10	..	..	..	..	11.0	47
macarellus	1	4	18	12	1	..	..	..	11.2	36

GILLRAKERS: LOWER LIMB

	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	$\bar{x}$	n
punctatus	..	..	1	..	9	21	41	25	22	14	5	2	1	..	1	36.8	142
tabl	2	17	24	4	..	..	..	..	..	..	..	..	..	..	..	31.6	47
macarellus	..	..	1	1	1	3	6	5	9	10	..	..	..	..	..	37.1	36

a single row along most of jaw; teeth in both jaws decreasing in extent with growth. Palatine teeth only on anterior head of bone, variable in extent and number. Vomerine teeth present laterally as a patch on head of vomer and medially in a single row or narrow band on shaft of vomer; the teeth very variable in number and extent of coverage in specimens of similar size, but generally increasing in both with growth, as is shown by the following selected examples comparing number of teeth with body length:

standard length	head of vomer	shaft of vomer
65	10	3
73	14	5
165	16	8
183	24	5
184	4	1
201	56	29
213	54	19
217	24	4
351	60	36
360	160	38.

Tongue teeth usually in a narrow band, slightly broader posteriorly, or a single row in some specimens; the band or row mainly on posterior part of tongue, and generally but variably decreasing in number of teeth and size of band with growth; no teeth on tongue of the 360 mm specimen from St. Helena, but a short, distinct band present in the 351 mm specimen.

Pigmentation. Adults originally preserved in 10–15% formalin and maintained in 40% isopropanol. Dorsal part of snout and head and dorsal third to half of body dark, the dark on head extending down slightly onto operculum and preoperculum; a dark opercular spot; tips of jaws, isthmus, branchiostegal membranes, and inner surface of operculum dusky; inner surface of lower jaw and dorsal surface of tongue dark to dusky. Dark pigment on dorsal part of body extending into a dark oval area over fleshy part of caudal peduncle and fin, this area encompassing the terminal scutes and scales of lateral line; ventral part of body clear. Rays of spinous and soft dorsal fins dusky, rays of other fins less dusky, especially pelvic fin rays; interradial membranes mostly clear. Peritoneum clear.

Coloration. Kodachrome slide (35 mm) of a freshly caught specimen from OREGON station 4843: Top of head dark, grading into metallic blue along back and upper part of body, this blue on body changing into dusky silver about one-half way down body with a silvery or white belly; most of straight part of lateral line bright silver, especially scutes; most of head silvery with dorsal part of snout very dark and upper jaw dusky. First dorsal fin with spines dark, membranes clear. Second dorsal fin with membranes clear, rays dark with tips of anterior 10 or so rays and the finlet tinged with red. Caudal fin bright red. Anal fin clear. Pectoral fin slightly dusky on dorsal part. Pelvic fins apparently tinged with red.

Size. The largest specimen examined (BMNH 1910.9.9.18) is from St. Helena, 360 mm SL, *ca.* 410 mm or 16.1 in. TL, and weighs 558 gms. (1.2 lbs.). The largest specimen examined from the western Atlantic (TABL 103024), 219 mm SL, *ca.* 332 mm or 13.1 in. TL, weighs 115 gms. (0.25 lb.).

Distribution. Currently known only from 55 specimens of 11 collections—from off North Carolina, off the east coast of southern Florida, off Colombia, off

TABLE 4

Frequency distributions of numbers of scales and scutes in the lateral line for the three western Atlantic species of *Decapterus*.

CURVED LATERAL LINE: SCALES

	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	x̄	n
punctatus	1	1	2	5	6	20	16	15	13	22	18	26	10	8	6	13	9	7	8	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	46.9	208
tabl	..	..	.	..	.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	1	2	4	9	3	7	7	4	6	2	2	1	..	..	..	1	..	68.0	50
macarellus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	3	7	2	3	5	2	2	3	1	..	3	72.9	33

CURVED LATERAL LINE: SCUTES

	0	1	2	3	4	5	6	x̄	n
punctatus	10	24	50	69	38	13	1	2.7	205
tabl	51	.	..	..	..	..	..	0.0	51
macarellus	39	..	..	..	..	..	..	0.0	39

STRAIGHT LATERAL LINE: ANTERIOR SCALES

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	x̄	n
punctatus	205	2	1	.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	.	..	..	..	..	..	..	..	..	..	.	..	..	0.0	208
tabl	8	4	9	3	9	8	7	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3.2	49
macarellus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	2	1	2	5	2	1	3	..	4	8	3	1	1	1	26.3	35

STRAIGHT LATERAL LINE: SCUTES

	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	x̄	n
punctatus	..	..	..	..	..	..	..	..	..	1	2	6	14	17	33	42	38	30	14	2	5	..	..	1	..	..	38.2	205
tabl	..	..	..	.	..	..	..	..	..	..	..	3	1	5	4	6	7	13	3	3	3	2	..	..	..	1	39.2	51
macarellus	1	1	1	6	8	3	4	7	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	28.0	35

TOTAL LATERAL LINE (excluding peduncle scales)

	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	x̄	n
punctatus	2	..	..	4	8	6	5	11	20	19	21	20	17	13	12	21	11	7	4	4	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	88.1	208
tabl	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	2	5	5	5	7	5	5	7	1	2	1	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	110.6	50
macarellus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	2	..	2	..	4	6	2	2	4	4	1	1	1	126.8	30

TABLE 5

Body proportions (in percent of standard length) of the three western Atlantic species of *Decapterus* including two specimens of *D. tabl* from St. Helena (315–360 mm SL)

	tabl			*punctatus*	*macarellus*
Standard length (mm)	156–199	195–219	351–360	140–183	145–256
Total length	113–115	115–115	114	116–117	115–115
Fork length	104–106	106–107	106	106–108	106–107
Head length	27.5–29.0	29.5–31.2	28.8–30.0	25.5–27.5	25.2–26.8
Eye diameter	6.5–7.4	7.1–8.4	7.1–8.5	7.1–7.9	5.9–6.9
Snout length	8.3–10.4	9.7–11.0	9.4–10.7	7.3–9.1	8.3–9.4
Postorbital head	11.8–13.0	11.2–12.9	11.4–11.9	10.2–12.3	10.5–11.4
Upper jaw	9.2–9.9	9.8–10.1	10.0–10.3	8.3–8.6	7.8–8.5
Maxillary depth	2.8–3.4	3.1–3.5	3.1–3.2	2.9–3.2	2.7–3.1
Depth D1 to D2	17.0–20.2	17.4–20.9	20.1–20.2	18.8–22.0	17.4–19.2
Depth D2 to A1	16.5–20.0	17.2–19.1	19.8–20.0	19.5–23.1	17.3–18.9
Depth max. vert.	16.7–19.8	17.4–19.4	19.5–19.7	18.8–23.0	16.1–18.9
Pectoral length	16.4–18.1	19.8–22.2	18.8–19.4	18.2–23.5	15.3–17.5
Pelvic length	10.5–12.1	11.9–12.9	11.4–12.9	11.0–13.0	9.9–12.1
D. spine (longest)	12.4–13.9	12.6–15.0	13.2–13.8	12.4–15.6	10.3–13.6
D. softray (longest)	10.2–11.2	11.3–12.8	10.9–11.4	11.5–12.4	8.9–9.5
A. spine (longest)	2.9–3.7	2.8–3.4	2.9–2.9	4.2–4.6	3.2–3.8
A. softray (longest)	8.8–9.5	8.8–10.2	9.5–9.9	10.1–11.3	7.5–8.7
Lateral-line ratio	0.61–0.91	0.68–0.98	0.67–0.81	0.92–1.16	0.79–1.05

Venezuela, and from St. Helena. The two smallest specimens (65–73 mm) were taken at the surface; the larger specimens from Florida (117 mm) and Colombia (149–219 mm) were taken in nets fishing on the bottom at 75 to 120 fathoms; the specimen from off Venezuela (194 mm) was taken in a bottom net fishing at 220 fathoms. Although the specimens taken in bottom-fishing nets may have been taken while the nets were descending or ascending, these records indicate that in its adult stage this a more offshore and deeper living species than *punctatus* or *macarellus*.

Holotype: USNM 202744, 202 mm SL, OREGON station 5711, Colombia, 12° 13′ N, 72° 29′ W, 13 October 1965, 40-ft. fish trawl, 113 fathoms.

Paratypes: TABL 103026, (1 specimen) 194 mm SL, OREGON station 4453, Venezuela, 10° 54′ N, 67° 08′ W, 12 October 1963, 40-ft. flat trawl, 220 fathoms. TABL 103025, (1) 180 mm, OREGON station 4844, Colombia, 11° 06.7′ N, 74° 30′ W, 17 May 1964, 40-ft. flat shrimp trawl, 100 fathoms. TABL 101476, (13) 149–199 mm, (2, cleared and stained) 156–185 mm, (1, in Ionol) 183 mm; USNM 202746, (4) 158–184 mm; AMNH 26940, (1) 175 mm; ANSP 109542, (1) 177 mm; BMNH 1968.4.19.1, (1) 158 mm; CAS ....., (1) 180 mm; FMNH 74536, (1) 182 mm; MCZ 45996, (1) 181 mm; MNHN 1968–3, (1) 177 mm; RMHN 25916, (1) 180 mm; UMMZ 187315, (1) 177 mm, OREGON station 4843, Colombia, 11° 08.5′ N, 74° 29′ W, 17 May 1964, 40-ft .flat shrimp trawl, 100–120 fathoms. TABL 103027, (2) 188–190 mm, OREGON station 4913, Colombia, 12° 09′ N, 72° 47′ W, 1 June 1964, 40-ft. flat shrimp trawl,

100 fathoms. TABL 101723, (1) 197 mm, OREGON station 4914, Colombia, 12° 08′ N, 72° 41′ W, 1 June 1964, 40-ft. flat shrimp trawl, 75 fathoms. TABL 103024, (12) 195–219 mm; USNM 202745, (2) 202–204 mm, OREGON station 5711, collected with holotype. TABL 103004, (1) 73 mm, MISTY II, cruise 24, station 81, Florida, 25° 31′ N, 79° 52′ W, 24 March 1967, 100-ft. purse seine, surface to 17 ft. TABL 103003, (1) 65 mm, MISTY II, cruise 25, station 83, Florida, 25° 35.3′ N, 80° 02′ W, 8 April 1967, 100-ft. purse seine, surface to 17 ft. USNM 155271, (1) 117 mm, PELICAN station 204–4, Florida, 28° 56′ N, 80° 01.5′ W, 29 March 1940, 10-ft. try net, 100 fms.

Three specimens identified as *tabl* are not designated as paratypes, and the species description was not based on these three. One is partially digested, and was part of the stomach contents of a *Coryphaena hippurus*, caught trolling off North Carolina. The other two are very large specimens from St. Helena, and as yet there are no specimens of comparable size from the western Atlantic with which to compare them.

Etymology. An "arbitrary combination of letters" (International Code of Zoological Nomenclature, Appendix D. V.), *TABL*—The Tropical Atlantic Biological Laboratory of the Bureau of Commercial Fisheries established on Virginia Key, Miami, Florida, October 1965—to be treated as an indeclinable noun.

Relationships. The two specimens identified as *tabl* from St. Helena (BMNH 1868.6.15.14, 1910.9.9.18) are similar to four specimens (BMNH 1867.10.8.42–43, 1946.5.23.1–2) of "Stonebrass" (*fide* Cadenat and Marchal 1963: 1268) from St. Helena in numbers of lateral-line scales, but differ in having fewer gillrakers (11–12 + 31–32 *vs* 14–16 + 41–44).

D. tabl differs from *punctatus* in the western Atlantic and at Bermuda in lacking spots in the curved lateral line; in averaging more rays in the pectoral fin and fewer gillrakers (Table 3); in the shape of the lateral line (Fig. 5 *vs*. Fig. 4); in having more scales in the lateral line (Table 4); and in having a longer head and upper jaw, a shorter anal fin first spine, and a lower lateral-line ratio (Table 5).

D. tabl differs from *macarellus* in the western Atlantic and at Bermuda in averaging fewer anal fin softrays and lower limb gillrakers (Table 3); in the shape of the lateral line (Fig. 5 *vs*. Fig. 6); in having more lateral-line scutes, fewer anterior scales in the straight lateral line, and fewer total lateral-line scales Table 4); and in having a longer upper jaw, a longer dorsal fin first softray, and a longer anal fin first softray (Table 5).

DECAPTERUS PUNCTATUS (CUVIER)

ROUND SCAD

FIG. 2

Scomber Hippos (*non* Linnaeus): Mitchill 1818: 246 (misidentification; speci-

men from New York market, more than 8 in. long; specimen probably not now extant).

Caranx punctatus Cuvier, 1829: 208 (replacement name for Mitchill's 1818 *Scomber Hippos*; type specimen and type locality that of Mitchill's specimen).

Caranx punctatus Agassiz in Spix, 1831: 108, pl. LVI, fig. 2 (type locality Atlantic Ocean, probably along Brazil; two type specimens 5½–6 in. long, originally reported in Museo Monacensi, probably not now extant).

Caranx punctatus Cuvier in Cuvier and Valenciennes 1833: 38 (type locality Martinique; type specimens 6 pouces long, originally in MNHN).

Synonymy. In 1815, Mitchill illustrated (pl. V, fig. 5) *Decapterus punctatus*, but did not give a description or scientfic name, only captioning the figure as "Hippos Mackerel." Mitchill described the species in 1818 (p. 246–247), erroneously applying the name *Scomber Hippos* of previous authors, which applies to *Caranx hippos* (Linnaeus). Cuvier (1829: 208) wrote the following in a footnote: "*Car. punctatus*, N., nommé *Scomber hippos* par Mitch., Trans. de New-York, I, v, 5; mas qui n'est pas l'hippos de Linnaeus." Agassiz in Spix (1831: 108, pl. LVI, fig. 2) described and illustrated the species as *Caranx punctatus*, giving himself as author of that name and mentioning the pre-Linnean work on Brazilian fishes by Marcgrav, but not referring to the work of Mitchill or Cuvier (above). Cuvier in Cuvier and Valenciennes (1833: 38), described the species as "*Caranx punctatus*, nob.," based on specimens collected by M. Plée at Martinique, again citing Mitchill, but not mentioning his own earlier reference or that of Agassiz. Most authors have cited Agassiz as the author of the species, and in doing so, Bertin and Dollfus (1948: 10) listed Cuvier's 1829 use of *Caranx punctatus* as a *nomen nudum*. Cuvier (1829) is the author of the species, however, because his *Caranx punctatus* is the earliest available name. It satisfies the necessary articles of the International Code of Zoological Nomenclature: 10; 11 a, b, c, g; 12; 16 a, i and iii. Thus, the species was technically described as a new species three separate times, each with the identical binomial.

Diagnosis. A *Decapterus* with 10–14 spots on scales in a relatively short curved lateral line; scutes in posterior part of curved lateral line usually 1–5; scutes in straight lateral line 32–46; total lateral-line scales and scutes (excluding peduncle scales) 77–98; lateral-line ratio 0.92–1.16; gillrakers 11–16 + 32–44; pectoral softrays 18–20; vertebrae 10 + 15.

Description. Frequency distributions for significant meristic characters for western Atlantic specimens are shown in Tables 3 and 4. Ranges for significant morphometric characters of western Atlantic specimens are shown in Table 5. Total scales and scutes in the curved lateral line range 40–59, mean 49.5. Total scutes in the curved and straight lateral line range 34–49, mean 41.0. Straight lateral-line scales on the peduncle range 2–6, mean 3.5. Total scales and scutes in the lateral line (including peduncle scales) range 83–101, mean 92.0. Gillrakers over the hypobranchial range 11–16. Rudimentary gillrakers on the an-

terior ends of upper and lower limbs are usually absent, rarely one present on either limb. Vertebrae are 10 + 15 (212 specimens). The ultimate softray (finlet) of dorsal and anal fins is connected by a membrane to penultimate softray to about 45–50 mm SL. The curved lateral-line spots are concentrations of melanophores on the medial surface of pored scales in the lateral line; they are obviously forming on some specimens as small as 70 mm SL, and the usual complement of 12–14 spots is present on some specimens as small as 81 mm SL, but they are not apparent on other specimens as large as 119 mm SL (possibly due to inadequate preservation).

Size. The largest specimen examined from the western Atlantic (TABL 101748) is 183 mm SL, 8.4 in. TL, and weighs 109 gms. (0.24 lb.).

Distribution. Along the Atlantic seaboard from Nova Scotia to Rio de Janeiro, Brazil; throughout the West Indies; Bermuda. Available records indicate that this areal distribution is continuous. Smaller juveniles are pelagic and apparently prefer oceanic rather than neritic water over the continental shelf, but they may occur inshore around oceanic islands. Juveniles have been taken as far as 270 miles offshore. They remain pelagic to a size of *ca.* 80 mm SL, although they may be caught in bottom-fishing trawls as small as 45 mm. Larger juveniles and adults may be taken at the surface and close inshore, but most of the records of this study were taken in bottom-fishing trawls that were fishing in depths of 5 to 50 fathoms; and a 123 mm specimen was taken in a trawl that fished the bottom at 200 fathoms. Because these were not closing trawls and they could have caught the scad somewhere between the surface and the bottom, the depth records are uncertain. I presume for the records out to 50 fathoms that the fish were on or near the bottom, but, lacking additional records, I suspect that the 200-fathom record may represent a fish that was taken in midwater or above.

Relationships. D. punctatus has been distinguished from *tabl* under the account of that species. It differs from the other western Atlantic species, *macarellus*, in having spots along the curved lateral line and in the shape of the lateral line (Fig. 4 *vs.* Fig. 6); in having fewer pectoral fin softrays (Table 3); in having one more caudal vertebral centrum (15 *vs.* 14); and in being deeper-bodied between the second dorsal and first anal spine origins, and in having a longer pectoral fin, longer dorsal and anal fin lobes, and a longer anal fin first spine (Table 5).

D. punctatus in the western Atlantic is quite variable in certain characters, especially in numbers of lower limb gillrakers and scales in the curved lateral line; but these variations do not appear to be correlated with geographic location or sexual dimorphism. Specimens from Bermuda are considered to be conspecific with *punctatus* from the western Atlantic, although the small sample from Bermuda averaged slightly fewer softrays in the dorsal fin (31.1) and anal fin (27.1), and more gillrakers on the upper limb (14.4) and lower limb (38.9).

I consider the western Atlantic and Bermudan *punctatus* to be specifically distinct from its cognate along West Africa, at St. Helena, and other eastern Atlantic islands. The eastern Atlantic species averages more scales in the curved lateral line (46–63, mean 54.1, 59 specimens) and fewer scutes in the straight

lateral line (30–38, mean 34.1, 62 specimens). This difference is reflected in the longer curved lateral line and correspondingly shorter straight lateral line of the eastern Atlantic species, which has a lesser lateral-line ratio (0.71–0.94) than *punctatus*.

I consider the valid name of the eastern Atlantic species that is cognate to the western Atlantic *punctatus* to be *Decapterus sanctaehelenae* (Cuvier), based on *Caranx sanctaehelenae* Cuvier in Cuvier and Valenciennes (1833: 37), type locality St. Helena. Cuvier stated (p. 38), "Nos individus sont longs de neuf pouces"—this equals *ca.* 243 mm TL. Bertin and Dollfus (1948: 10) reported 14 specimens from St. Helena in the Paris Museum to be syntypes of "*Caranx sanctaehelenae*" (which they considered to be a junior synonym of *Decapterus punctatus*). I examined these specimens in October 1965: MNHN 5852, 3 specimens, 151, 163 and 175 mm SL, collected by Quoy and Gaimard; MNHN 5948, 2 specimens, 163 and 172 mm SL, collected by Quoy and Gaimard; MNHN 5946, 8 specimens, 74–101 mm SL, collected by Dussumier; and MNHN 5947, 1 specimen, 192 mm SL and *ca.* 220 mm TL, collected by Lesson and Garnot. I herein designate as the lectotype of *Caranx sanctaehelenae* Cuvier the largest specimen from MNHN 5852, 175 mm SL, bearing a numbered tag tied to the lower jaw. In doing this, I have to disagree with Cadenat and Marchal (1963:1269), who considered that these specimens were not syntypes of Cuvier's *sanctaehelenae*, and applied that name to the "stonebrass" of St. Helena (a species that I diagnose as lacking spots in the curved lateral line, and having 14–16 + 41–44 gillrakers, 57–64 scales and no scutes in the curved lateral line, 34–38 scutes, and 95–103 total scales and scutes in the lateral line). By my interpretation, the St. Helena "stonebrass" lacks a valid scientific name.

DECAPTERUS MACARELLUS (CUVIER)

MACKEREL SCAD

FIG. 3

Caranx macarellus Cuvier in Cuvier and Valenciennes, 1833:40 (type locality Martinique; lectotype 256 mm SL, paralectotype 131 mm SL, both MNHN 5850).

Caranx Jacobaeus Cuvier in Cuvier and Valenciennes, 1833:42 (type locality Porto Praya, San Iago Id., Cape Verde Ids.; holotype MNHN 5849, 260 mm SL, *ca.* 295 mm TL).

Diagnosis. A *Decapterus* with a relatively long and low curved lateral line, lacking spots; 19–33 scales in anterior and 23–32 scutes in posterior part of straight lateral line; lateral-line scales and scutes (excluding peduncle scales) 119–133; gillrakers 9–13 + 32–39; pectoral softrays 21–23; vertebrae 10 + 14.

Description. Frequency distributions for significant meristic characters of western Atlantic specimens are shown in Tables 3 and 4. Ranges for significant morphometric characters of western Atlantic specimens are given in Table 5. Straight lateral-line scales on the peduncle range 6–13, mean 8.5. Total scales

and scutes in the lateral line (including peduncle scales) range 126–140, mean 134.0. Gillrakers over the hypobranchial range 11–14. Rudimentary gillrakers on the anterior ends of the upper and lower limbs are usually absent, rarely one present on either limb. Vertebrae are 10 + 14 (21 specimens). No spots occur on scales of curved lateral line.

Size. The largest specimen examined was the lectotpye (MNHN 5850), 256 mm SL, *ca.* 295 mm or 11.6 in. TL. The next largest specimen examined from the western Atlantic (TABL 101731) is 247 mm SL, *ca.* 289 mm or 11.4 in. TL, and weighs 208 gms. (0.46 lb.).

Distribution. In the western Atlantic, known from (but not continuously) Nova Scotia to Fernando de Noronha off northeastern Brazil; at Bermuda; and in the eastern Atlantic at the islands of Cape Verde, St. Helena, Ascension, and in the Gulf of Guinea. Locations of specimens I have examined in the western Atlantic are quite dispersed: from the West Indies in the northern Bahamas, Porto Rico, Virgin Islands, Dominica, Martinique, and Grenadines; Curacao and Albuquerque Key in the Caribbean; and continental records from British Guiana, Colombia, Florida (off Miami), and Massachusetts. The available records suggest that this is primarily a pelagic and offshore species, and it is probably associated more with oceanic islands than continental areas. Three collections of the smallest juveniles were taken at the surface in the Gulf Stream off Miami. Larger specimens have been taken inshore with beach seines and pound nets (West Indies and Massachusetts); and the largest specimens examined were taken at the surface with a fish pump over 18 fathoms of water. One specimen was speared at a depth of *ca.* 12 fathoms over coral rubble. I have records of one specimen that was caught in a trawl on the bottom at 36–40 fathoms and another from 270 fathoms, but I assume that the latter, at least, was caught when the net was fishing well above the bottom.

Relationships. D. macarellus from the western Atlantic has been distinguished from *tabl* and *punctatus* under the accounts of those two species.

Based on available study specimens and literature accounts, I consider that only a single species of the *macarellus*-form exists in the Atlantic Ocean, and I tentatively record this as specifically distinct from a similar form or forms in the Indo-Pacific. Most recent authors have subscribed to the first consideration, but some have variously considered the Atlantic form to be the same species, or subspecies, or one or more distinct species from the Indo-Pacific named forms. Bertin and Dollfus (1948: 14–15) list the nominal Indo-Pacific species that have been included in synonymy of *macarellus*.

SPECIES AGGREGATIONS

In all of the individual collections of western Atlantic *Decapterus* I have examined, only a single species was present in each, with the exception of four collections. These exceptions were all from collections made off Miami, Florida, by James K. Dooley with a 100-ft. purse seine in the Gulf Stream *Sargassum* community. From April 1966 into May 1967, 102 such collections were made;

and 14 of those caught *punctatus* juveniles (34–80 mm SL). At two of these stations, both *punctatus* and *macarellus* were taken. At one station, both *punctatus* and *tabl* were taken. One station contained all three species: *punctatus* (89 specimens) 43–53 mm SL, *macarellus* (3) 50–62 mm, and *tabl* (1) 73 mm.

SPECIMENS EXAMINED

Decapterus tabl. North Carolina: (1) *ca.* 113 mm, TABL 103203, 34° 34′ N, 75° 19′ W, *ca.* 60 mi. E Cape Lookout, in stomach contents of female 675-mm FL *Coryphaena hippurus* (Linnaeus), caught surface trawling, 11 July 1954, GILL cr. 7, between reg. sta. 74 and 80. St. Helena: BMNH 1868.6.15.14, (1) 351, coll. J. C. Mellis; BMNH 1910.9.9.18, (1) 360, coll. J. T. Cunningham. Holotype and 51 paratypes (listed under the species account).

Decapterus macarellus. Massachusetts, USNM 13660, 20639, 38279, 38306, 82252, 84030. Florida Atlantic, TABL 103016, 103017, 103018. Colombia, TABL 103202. Guyana, BMNH 1961.9.1.21. Curacao, ANSP 52024. Alburqueque Key, ANSP 74159. Northern Bahamas, BMNH 1934.8.2.1–2; ANSP 108580. Porto Rico, UMML 2264, 8163; TABL 101730. Martinique, MNHN 5850 (lectotype 256 mm SL, paralectotype 131 mm SL). Dominica, TABL 101724. Cannouan, TABL 101731, 103319. West Indies, USNM 108388. Bermuda, FMNH 48341, 48411, 48412, 48535.

Decapterus punctatus. Massachusetts, USNM 16239, 58884, 58921, 77796, 120566, 128647. Delaware, USNM 187175. Western Atlantic from off North Carolina to off Georges Bank, USNM 189250, 189261, 189262, 189263, 189265, 189266, 189270–3, 189283, 189285, 189286, 189288, 189289, 189359. North Carolina, USNM 53117, 198996, 199019, 199026, 199034; TABL 101477, 101729, 101745, 101746, 101747, 101748, 101749, 101750, 101751, 101767, 101768, 101769, 101826, 101832, 102572, 102573, 102574, 102575, 102577, 102578, 102579, 102580, 102581, 102582, 102583, 102584, 102585, 102586, 102587, 102588, 102976, 102977, 102981, 102982, 103117. South Carolina, USNM 199013, 199016, 199024, 199032; TABL 101728, 101753, 101765, 101775, 101776, 101777, 101778, 101819, 101833, 102983, 102985, 102986, 102987, 102988, 103114, 103120. Georgia, USNM 199018; TABL 102570, 101727, 101773, 101779, 101784, 102978, 102980, 103119, 103127. Florida Atlantic, USNM 198995, 199012, 199042; TABL 101752, 101764, 101771, 101772, 101780, 101781, 101782, 101783, 101796, 101797, 101815, 102064, 102979, 103005, 103006, 103007, 103008, 103009, 103010, 103012, 103013, 103015, 103021, 103022, 103023, 103113, 103118, 103121, 103124, 103125, 103126. Florida Straits and Florida Keys, USNM 198973; TABL 101758, 101759, 101760, 101761, 103116. Florida Gulf, USNM 198976, 199020, 199021, 199022, 199029, 199037; FSBC 2689, 2728, 2927, 2968, 3069, 3434, 3597, 3700, 60–24, 62–56, 63–35; TABL 101785, 101999, 102062, 103122. Alabama, USNM 199015, 199017. Louisiana, TABL 101787. Texas, USNM 199014; TABL 101725, 102971. Mexico, Campeche, USNM 198993; TABL 101744, 102569. Mexico, Yucatan, TABL 102571. Honduras, TABL 102710. Costa Rica, TABL 101726. Panama,

USNM 80037. Colombia, TABL 102568, 102972, 102973, 102974, 102975. Venezuela, TABL 102566, 102567, 102970. Guyana, BMNH 1961.9.1.11–20; TABL 101786, 102964, 102965, 102966, 102967. French Guiana, USNM 159822. Brazil, Maranhao, TABL 102969. Brazil, Bahia, USNM 43352: BMNH 1862.1.30. 41. Brazil, Rio de Janeiro, BMNH 1903.6.9.73, 1923.7.30.133–6. Blake Plateau, TABL 103115. Northern Bahamas, ANSP 102157; TABL 102984, 103123. Cuba, USNM 6028. Jamaica, LACM 5445. Porto Rico, USNM 126429, TABL 102565. Virgin Islands, TABL 102968. Anguilla Id., TABL 101789. St. Martin Id., TABL 101791. Dominica, TABL 101790. Martinique, TABL 103318. St. Lucia, TABL 101732, 103321. St. Vincent, BMNH 1931.12.5.306–7. Bequia, TABL 101733, 103313, 103317, 103320. Canouan, TABL 103312. Carriacou, TABL 103309. Grenada, TABL 101792, 103310, 103311. Barbados, TABL 103315. Tobago, USMN 190507, BMNH 1920.12.22.41–44, 1922.6.22.56; TABL 103314. Trinidad, TABL 103316. Eastern Caribbean, TABL 102564. Bermuda, USNM 10334, 21365, 160980, 178417, 251937; BMNH 1872.8.28.37, 1879.10.9.47; ANSP 52337–8.

GENERIC RELATIONSHIPS

The morphological characteristics and limits of the genus *Decapterus* are not as yet adequately defined. The same is true for the genus *Caranx* Lacépède 1802, and other associated genera. Precise definition and restriction and species allocation must await thorough analysis of all species concerned.

In their review of the genus, Bertin and Dollfus (1948) included 7 species in the genus *Decapterus*, but 2 of these relate to other genera: 1) *Caranx suareus* Risso in Cuvier and Valenciennes, 1833:33 (type locality Nice, France; holotype 435 mm SL, MNHN B. 869) with the following included as a junior synonym, *Decapterus longimanus* Norman 1935:255 (type locality Tristan de Cunha; holotype 412 mm SL, BMNH 1935.5.2.3). Both of these nominal species relate to the genus *Trachurus* Rafinesque, 1810, and *suareus* was correctly related to *Trachurus* by Blanc and Bauchot (1961:491). 2) *Decapterus normani* Bertin and Dollfus 1948:21 (type locality Madagascar; holotype 254 mm SL, MNHN 47–7). This is a junior synonym of *Atule mate* (Cuvier in Cuvier and Valenciennes 1833:54), as defined by Williams (1958:378).

Bertin and Dollfus (1948:9) included as one of their 7 species of *Decapterus*, *Caranx rhonchus* Geoffrey St. Hilaire 1809. I concur with this generic placement, although Blanc and Bauchot (1961:491) considered *rhonchus* to be a species of *Caranx*.

Suareus furnestini Dardignac and Vincent in Furnestin *et al* (1958:445), described as a new genus and species related to *Trachurus*, was correctly included in *Trachurus* by Blanc and Bauchot (1961:491).

Two other genera form a closely-related complex with *Decapterus*—*Trachurus* and *Selar* Bleeker 1850. [I follow Ginsburg (1952:83) in recognizing *Trachurops* Gill 1862, as a junior synonym of *Selar*.] These 3 genera can be distinguished from other Caranginae by the following combination of characters: 1) Scales

present on all 4 pairs of opercular bones, on interorbital region, and usually on maxillae; chest completely scaled. 2) First 2 anal spines of similar length, the first usually longer, or sometimes slightly shorter, than the second; the second definitely not much longer than the first. 3) Teeth usually present in a band on tongue, on both head and shaft of vomer, and frequently on head of palatines; small teeth present on both jaws, either in a single row or a narrow band. 4) No bilateral, paired keels on caudal peduncle.

The three genera can be distinguished by the following combination of characters.

1a) Pored scales in curved lateral line expanded dorsoventrally, scute-like, *Trachurus*.

1b) Pored scales in curved lateral not so expanded, about as long as high, *Selar* and *Decapterus*.

2a) A single-rayed finlet present at end of dorsal and anal fins, or the terminal finray having a greater distance between it and penultimate ray than between more anterior rays, *Decapterus* and *Trachurus*.

2b) Terminal fin ray of dorsal and anal fins close to penultimate ray, not greatly displaced or forming a finlet, *Selar*.

3a) Bilateral dorsoposterior accessory lateral lines converging and ending at end of supraoccipital crest, *Decapterus*.

3b) Accessory lateral lines extending to origin of first dorsal fin or beyond, *Trachurus* and *Selar*.

4a) Two fleshy papillae on shoulder present or lacking, if present neither especially enlarged, *Decapterus* and *Trachurus*.

4b) Two fleshy papillae present on shoulder girdle, the lower enlarged with a furrow formed at its ventral surface, *Selar*.

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